5-2005

Humic and Fulvic Acids: Effects on Plant Nutrition and Growth

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HUMIC AND FULVIC ACIDS: EFFECTS ON PLANT NUTRITION AND GROWTH

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science
(Crop Physiology)

Approved:

UTAH STATE UNIVERSITY
Logan, Utah

2005

ABSTRACT

Humic and Fulvic Acids: Effects on Plant Nutrition and Growth

by

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Utah State University, 2005

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Humic substances are reported to improve plant growth and nutrient uptake, with iron the most studied nutrient. The most common forms of iron in soils are iron oxides, which are stable under aerobic conditions and unavailable for plant uptake. Iron-deficient plants become chlorotic, which reduces growth and yield. To determine if humic substances can reduce iron chlorosis, five commercially available organic acids were tested on maize grown in sand columns at high pH. The dry granular humic acid from Aldrich Chemical Company applied at 84.4 g/liter of sand by volume (5% by mass) and 1 g/liter added with irrigation water, significanlty reduced iron chlorosis ($p<0.0001$). It also increased fresh mass by 39% and improved root growth. The other products, applied at 50 µl/liter did not significantly affect chlorosis or plant growth.

A second objective was to determine if humic substances improve plant growth and yield. The effects of 11 commercially available dry granular products on tomato growth were studied in soil columns in a greenhouse. A product from Horizon Ag

Products (Modesto, CA), DGX FeZnMn Blend at a rate of 44.8 kg/ha (40 lbs/acre), significantly improved root growth, but not shoot growth or yield.

In another study, 10 commercially available liquid products from Horizon Ag Products were tested. Treatments were applied at 50 ml/liter mixed with irrigation water. Treatment BA6.6% increased fruit number, fruit dry mass and plant dry mass. Treatments QH6.6% and Charger increased plant dry mass. Treatments QH6.6%, Hydra-Hume6% and F-6000 increased fruit dry mass. However, this was an extremely high application rate. When the study was done at an economical rate of 4.6 μl/liter, there were no significant beneficial effects. Most application rates reported in the literature are considerably higher than economic rates applied in the field. The results of these studies indicate some effects on plant nutrition and growth when applied at high rates but limited effects when applied at low (economic) rates.

(160 pages)

ACKNOWLEDGMENTS

There is an end to a master's program, even though I claimed to be on the fifty year plan, and I would like to thank the many people who helped out along the way. First I would like to thank my wife, Naomi, for her patience, support, and understanding. I would also like to thank my extended families for their encouragement and thoughtfulness.

A very large thank you to Dr. Bruce Bugbee as my major advisor for giving me the chance to earn a master's degree, and for all his time and thought that helped and improved these projects. Bruce also spent plenty of time teaching me how to analyze data and write appropriate reports, THANKS again Bruce. Thank you to my other committee members, Dr. Paul Grossl and Dr. Mike Amacher, for their time and expertise contributed to this research.

Thanks to Horizon Ag Products for funding this research and their input and knowledge. Specific thanks to Ken Day who sought out the Crop Physiology Laboratory as a research facility, and his input into these studies. Thanks to Craig Muma, president of Horizon Ag Products, for his interest, support, and open-mindedness of the research projects done here. I also thank Perry Bosshart who kept me on my toes when analyzing the last of the data.

Thanks Julie Chard for helping in almost every aspect of this research, and for doing many side projects that improved the research. Thank you to the undergraduate workers who helped in many ways including: Nick Knighton, Emily Mills, Rob Hyatt, and Jason Holman, also to Robson Chaves who is a non-undergraduate lab assistant worker. Thank you also to Alec Hay for his insight and effort in constructing experimental systems.

Thank you to the other professors, Rich Koenig, Scott Jones, and Janis Boettinger, whose specialties and insights helped this project along the way.

Jason Tew

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CHAPTER 1

INTRODUCTION

HISTORY

In a review paper, Chen and Aviad (1990) gave a chronology on the importance of organic matter. They pointed out that man has known for hundreds of years that dark-colored soils were more productive than light colored soils, and that productivity was related to decaying organic organisms. They found research from 1699 showing that plants grown with water from different sources responded as follows: soil water extract > river water > well water, this also correlated with how yellow the water was. They also cite a work from 1808 in which a theory was laid out suggesting that humic substances directly affect plant nutrition, and are the only source of plants nutrients.

Chen and Aviad (1990) conclude their history in the early 1900's. Within the next hundred years it was shown that plants synthesize organic matter using CO_2 and water. In addition, it was found that minerals were vital for plant growth. This culminated in 1905 when it was shown that soil fertility could be maintained for several years using only mineral fertilizers. By 1920 humic substances had been shown to enhance the growth of various plant species in nutrient solutions. A proposed mechanism for this improved growth was that humic substances increase the solubility of mineral ions.

ORGANIC MATTER

Organic matter that has reached the stage of decomposition where it is no longer recognizable as plant, animal or microbial remains is called humus. Humic and fulvic acids are the water-soluble portions of humus. The non-soluble fraction is called humin. Humic Acid (HA) is soluble at pH > 2 while Fulvic Acid (FA) is soluble at any pH (MacCarthy, 2001). Together they are often referred to as humic substances. Humic substances can range in size from a few hundred to several hundred thousand atomic mass units (an atomic mass unit is the mass of one proton in an atom) (Gaffney et al., 1996). Fulvic acids are the smaller compounds and are usually less than 3000 atomic mass units. The soluble nature of these compounds leads to the widely held view that they are the active parts of soil organic matter (SOM). Both of these compounds are highly oxidized with fulvic acid being slightly more so (Figure 1).

The color change from fulvic to humic acid is obvious (Figure 2).

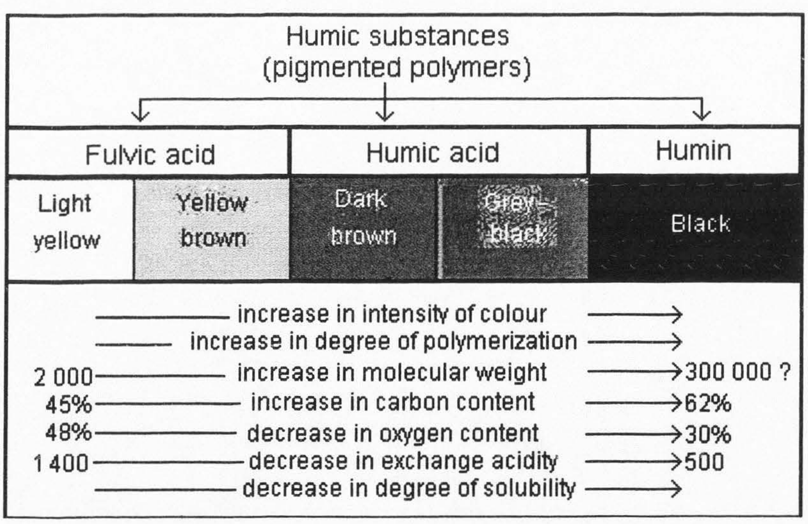

FIGURE 1. Chemical properties of humic substances (Stevenson, 1982).

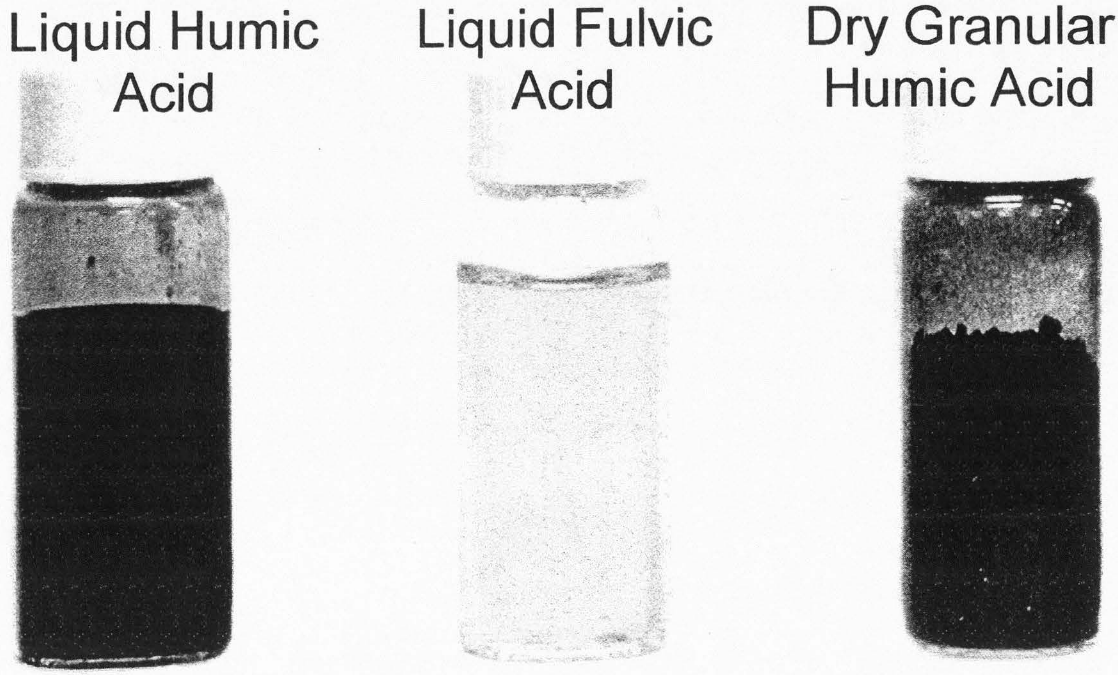

FIGURE 2. Appearance of three representative commercially available humic substances.

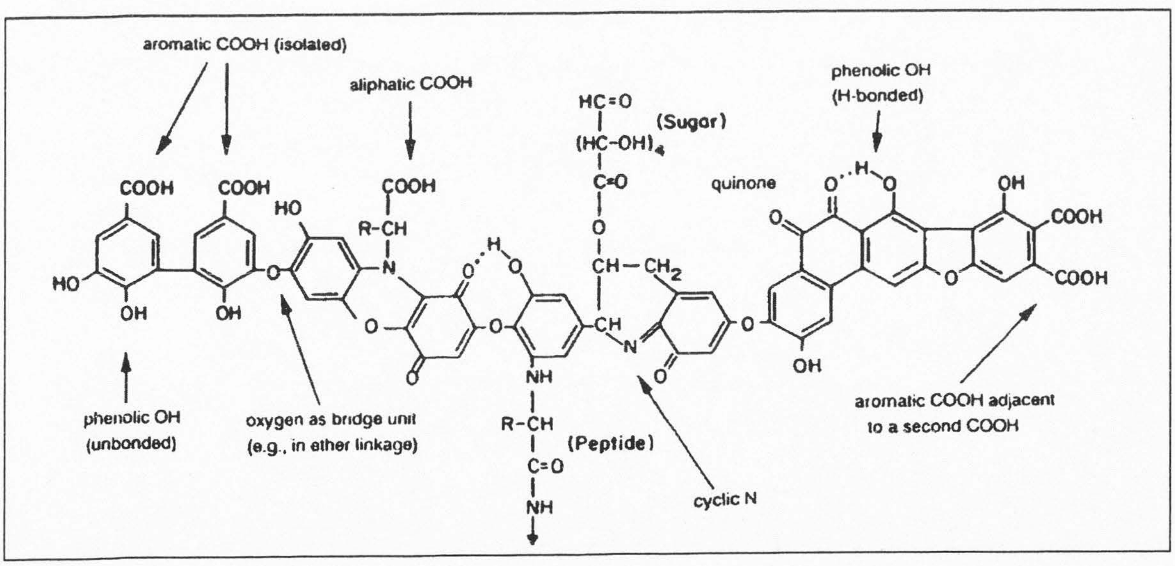

FIGURE 3. A hypothetical structure of humic acids, with the most common functional groups (Stevenson, 1982).

Like all soil organic matter these compounds are made of many interconnected aromatic rings with some aliphatic chains (Figure 3). Important functional groups on humic and fulvic acids include phenols, carboxyls, alcohols, methoxyls, hydroxyls and amides. Quinoid, ester and keto functional groups have also been identified by some researchers (Orlov, 1995).

The following effects have all been attributed to HA and/or FA: improved soil structure, better water use efficiency, increased plant growth, seed germination, seedling growth, root initiation, root growth and shoot development (Chen and Aviad, 1990; Nardi et al., 1996), increased soil Cation Exchange Capacity/acid-base buffering capacity (Cooper et al., 1998; MacCarthy, 2001) and mineral nutrition (Adani et al., 1998; Chen and Aviad, 1990).

Beneficial affects have not been consistently observed (Brownell et al., 1987). There are numerous papers that have found no effect, and some even report detrimental effects (de Kreij and Basar, 1995; Linehan, 1978). Presumably there are many research projects that aren't published because they fail to find beneficial effects. Among the papers that claim improved conditions, there are differences in the optimal amounts, source of material and preparation techniques of organic matter (Brownell et al., 1987; Gaffney et al., 1996; Tan, 2003). Specifically, a few people have claimed that humic substances attained from soil or water are fundamentally different than those extracted from coal (Malcolm and MacCarthy, 1986; Stevenson, 1979).

STUDIES ON SOIL STRUCTURE

There is strong evidence that organic matter has a beneficial effect on soil structure. Farmers have known for hundreds of years that having organic matter in the soil improves its productivity. Organic matter, including humic and fulvic acids, can improve soil structure and porosity by increasing soil aggregation, aggregate stability (Piccolo and Mbagwu, 1989, 1994), and soil fertility (Glaser et al., 2002). The large size of organic matter allows each molecule to bind several soil particles and increase flocculation and aggregate stability. Increased soil structure improves porosity, thus increasing the amount of air available to plant roots. One of the key factors to maximize crop production is improving the root zone environment (Chen and Aviad, 1990).

Increased soil aggregation and soil stability have been reported using cattle manure (Fortun et al., 1989; Mbagwu et al., 1990), sewage sludges (Mbagwu et al., 1990), pig slurry (Mbagwu et al., 1990), ryegrass (Watts et al., 2001), and lignite (coal) derived products (Piccolo et al., 1996). However, increased soil structure was not seen when peat moss was used (Watts et al., 2001).

Piccolo and Mbagwu (1990) observed that humic acids improve soil structure but fulvic acids do not. However, fulvic acids may have an additive effect on soil structure when used in conjunction with other organic matter (Fortun et al., 1989). Why does fulvic acid not improve soil aggregation when used alone? The difference between humic and fulvic acids aggregating ability could be related to their solubility. Linehan and Shepherd (1979) reported that fulvic acids are soluble in the field while humic acids are not, meaning in the field humic acids are more like humin than fulvic acids. In connection with this, fulvic acids used alone have been reported to coat clay particles, the major flocculating portion of soil, thus inhibiting aggregation (Piccolo and Mbagwu, 1990). It is possible that fulvic acids can coat clay particles and keep them in suspension and moving through the soil profile. This may partially explain why fulvic acids alone do not improve soil structure, while the non-soluble humic acids bind several clay particles together in an aggregate. The overall size of the acid might play a large role in it flocculating abilities.

The additive effects of fulvic acid on soil structure may be partially explained by the claim that the amount of aliphatic chains in organic matter are closely correlated to soil aggregate stability (Capriel et al., 1990) and fulvic acids have more aliphatic chains than humic acid or humin (Gaffney et al., 1996). This suggests that fulvic acids improve soil aggregation, but they must interact with other organic matter to increase soil structure. Based on this, adding both soluble and non-soluble organic mater into a soil system should result in greater flocculation than adding either fraction alone.

Organic matter appears to be especially beneficial in soils that are highly weathered and/or have low organic matter contents (Lee and Bartlett, 1976; Mbagwu et al., 1990; Pagliai et al., 1981). However, Spaccini et al. (2002) did not find a statistically significant effect of mustard meal, maize residues, coffee husks, cow dung and urea in a low organic matter, highly weathered soil, but application of these compounds was correlated to soil stability. Generally humic substances improve soil structure but there are exceptions.

STUDIES ON PLANT GROWTH

Humic materials have been observed to improve plant growth in maize (Lee and Bartlett, 1976; Tan and Nopamornbodi, 1979), teak (Fagbenro and Agboola, 1993), winter wheat, pepper, sugar beet (Chen and Aviad, 1990), tomatoes (Adani et al., 1998; David et al., 1994) and other crops. Humic acids tend to have greater effect on root growth than on the shoots (Adani et al., 1998; Chen and Aviad, 1990; Tan, 2003).

David et al. (1994) and others also found that too much humic acid reduces beneficial affects. This implies that identifying the right amount is important to maximize growth. Unfortunately, no single concentration of humic or fulvic acid has been recognized to produce the best results (Chen and Aviad, 1990). Lee and Bartlett (1976) found 5 ppm to be the most beneficial to maize while Tan and Nopamornbodi (1979) found 640 ppm best. Rauthan and Schnitzer (1981) found 100 to 300 ppm to be best for cucumbers. David et al. (1994) found that 1280 ppm helped tomato growth, but 640 or 2560 ppm did not improve growth. Fagbenro and Agboola (1993) also found that the beneficial effects of HA supplied to soils at 50, 500, and 1000 mg/kg (100, 1000, and 2000 kg/ha) changed from month to month. Their general trend was increased growth at all treatment levels. Sanchez-Sanchez et al. (2002) observed increased yield when fulvic acid was added with EDDHA at 35g/tree on lemon trees. Pilanali and Kaplan (2003) grew strawberry plants in a calcareous soil over two years, and found that a humic product applied at 100, 200, 300, and 400 kg/ha had no beneficial effects on plant growth or nutrient uptake.

A portion of the rate discrepancy can be attributed to different extraction methods, preparation procedures, stock concentrations and source materials. A majority of the research, done on humic substances, has been done in hydroponics where 300 to 500 ppm has generally been found to be optimum.

The rates used in this research were generally the suggested economical field rates recommended by Horizon Ag Products. An example is 3 to 45 kg/ha when using dry granular products (liquid products are 3 to 5 μl/liter). These rates are lower, and sometimes many times lower, than rates reported in the literature.

STUDIES ON SOIL CEC AND SALT BINDING

The large number of functional groups on humic and fulvic acids results in high CEC values (Pandey et al., 2000). This attribute allows them to bind water-soluble cations, many of which are plant nutrients, and hold them in the soil environment where they are available to plants and microbes. It is also possible that they may bind sodium prevent damage to the soil and plants.

The amounts applied to soils are small when compared to the over all soil organic matter. For instance a typical soil has two million kg/ha in the top 15 cm of soil. If that soil is two percent organic matter then there is 40,000 kg of organic matter per hectare. A dry granular product applied at 40 kg/ha, would increase organic matter as a percent of the soil mass by 0.002 percent (from 2.000 to 2.002). The CEC of humic products can be up to 5 times that of the bulk soil organic matter (CEC of 1500 compared to 300) if the applied product was 100% pure (which they never are). With this the increase in CEC would be 5 times larger than the increase in organic matter fraction (roughly 0.01 percent). This is not enough to remove significant amounts of sodium or other salts.

The corresponding change in organic matter CEC calculated assuming that the bulk organic matter has a CEC of 300 and the added product has a CEC of 1500. The CEC of the soil would change. Not enough to remove large amounts of sodium or other salts.

STUDIES ON IRON (FE) NUTRITION AND OTHER NUTRIENTS

The high cation binding ability of HA and FA allows them to chelate nutrients for plants. Researchers have looked at many plant nutrients; however, iron has been studied the most (Nardi et al., 1996). DeKock (1955) was one of the first researchers to report iron chelated by humic acids.

Iron is a difficult element to study because it is hard to keep in solution, especially in high pH soils (pH 7 to 8) (Barnard et al., 1992). Free iron quickly forms iron oxides or precipitates with phosphorus. Plants can thus be iron deficient even though most soils have ample total iron. Shaw (1994) found that dissolved humic substances keep iron in solution.

These compounds may keep iron in solution but does that mean that the iron is available for plant uptake? In hydroponics, Mackowiak et al. (2001) found that HA can complex iron sufficiently for plant growth. In addition, Adani et al. (1998) saw an increased iron level in plants using two humic acid products at 20 and 50 ppm in hydroponic solution. The first product was a soil extract while the second was lignite derived. Humic acid was able to do this even at high pH (Barnard et al., 1992; Lobartini and Orioli, 1988). In supplemental work done at the Utah State University research greenhouses, a fulvic acid improved iron availability over some common commercially available chelating compounds, but a humic acid was not effective (Appendix B).

Will humic substances have the same effect in a soil environment? Fagbenro and Agboola (1993) doing research in two soils (an alfisol and an oxisol) found increased iron uptake at a humic substance rate of 500 mg/kg; however, this was not the case at 50 or 1000 mg/kg. Sanchez-Sanchez et al. (2002) found that HA applied to calcareous soil in conjunction with EDDHA increased the plant uptake of iron.

In soil environments, free iron quickly forms iron oxides and iron hydroxides, which are stable and in soluble. Available phosphorus (P) binds to the surface of these iron compounds and becomes equally unavailable to plants. Humic and fulvic acids can bind to iron oxides and coat them; this should prevent phosphate from absorbing to their surface and increasing phosphorus availability to plants. This increased phosphorus availability was observed by Adani et al. (1998), Fagbenro and Agboola (1993), Lee and Bartlett (1976), and Shaw (1994).

Researchers have examined almost all other plant nutrients, but there are no consistent reports of increased uptake. In fact, Pilanali and Kaplan (2003) found that humic acid had no significant effect on N, P, K, Ca, Mg, Fe, Mn, or Cu content in strawberry plants over a two-year period, except for a significant decrease in the Zn concentration. This is one of the few long term studies conducted on humic compounds

Several researchers have studied the binding preferences of humic substances to some plant nutrients and other environmentally important metals:

Cu>Ni>Co>Zn>Mn (Rashid, 1974)

Hg>Fe>Pb>Cu, Al>Ni>Cr, Zn, Cd, Co, Mn (Kerndorff and Schnitzer, 1980)

Fe>Al>Cu>Zn>Ni>Co>Mg (Baruah and Upreti, 1994)

Cu>Fe>Cr>Mn>Ni>Hg>Pb>Cd (Misra et al., 1996)

Cu>Fe>Pb>Ni>Co>Cd>Zn>Mn>Mg (Pandey et al., 2000).

These results suggest that of the plant nutrients examined, Fe and Cu are best chelated by humic substances and the greatest impact on uptake should be for these two nutrients. An effect on phosphorus availability should accompany the iron change because of its strong association with iron.

SUMMARY

Although it is conventional wisdom in the agricultural community that organic matter improves plant production, the scientific community has been unable to obtain consistent results to answer the questions of how, why and when humic substances promote plant growth.

OBJECTIVES

The overall objective of this research was to quantify beneficial effects of humic and fulvic acids on crop growth and yield, and iron chlorosis by:

1. developing a method of getting good plant growth in inert sand columns
2. developing methods to get controlled iron chlorosis using ferrihydrite as and iron source
3. analyzing iron uptake by taking leaf chlorophyll content measurements.

Additional objectives for growth and yield studies were:

1. developing a soil column system for growing healthy plants in field like conditions through an entire plant life cycle (which is rarely done in humic substance research)
2. to use digital pictures to analyze early plant growth
3. to identify which organic amendment products are most beneficial to plant growth.

HYPOTHESIS

Hypothesis 1: Humic and fulvic acids will increase crop growth and yield.

Hypothesis 2: Humic and fulvic acids will chelate iron and increase its bioavailability and uptake.

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CHAPTER 2

THE EFFECT OF ORGANIC AMENDMENTS ON PLANT GROWTH AND IRON AVAILABILITY IN SAND COLUMNS

ABSTRACT

Humic substances can reduce iron (Fe) chlorosis when they are applied with other chelates or when the humic substance is iron enriched. This study was done to see if humic substances have the ability to chelate iron and solubilize ferrihydrite, a naturally occurring form of iron. Five commercially available organic acid products were tested on maize plants in a sand system at high pH (pH 7.3 to 8.0). A dry granular product from Aldrich Chemical Company was applied at a rate of 84.4 g/liter of sand (5% by mass) mixed in with the soil and also watered at 1000 mg/liter. This treatment significanlty reduced iron chlorosis, increased plant fresh mass 39%, and improved root growth. The other four products were liquid fulvic acids applied at 3.7 and 50 µl/liter of irrigation water in seperate experiments. These four products did not have significant benificial effects on the observed growth parameters. The rates used in the literature are higher than either of the rates used for the liquid fulvic acid products in this study.

INTRODUCTION

Iron chlorosis is particularly troublesome in alkaline (calcareous) soils. Chen and Aviad (1990) report increased chlorophyll content in peanuts grown in a highly calcareous soil when iron enriched peat was added. However, the iron was added with the organic amendment, so it is not completely clear what effect the iron addition had and what affect the organic matter had.

A few researchers have used a sand system to study the effects of humic substances on plant growth. One of these studies by Cooper et al. (1998) grew creeping bentgrass and applied a dry granular humic acid product. They observed increased root mass throughout the system and an overall increase in root length. In addition they noticed increases in P and Mg uptake.

In a system more similar to ours; Barnard et al. (1992) grew maize in quartz sand using five different iron treatments under two lime levels. They found that fulvic acid improved shoot mass at both lime levels. This increased mass did not correspond with an increase of iron concentration in the shoots. The roots had significant increases in growth and iron concentration, but only at the higher lime level. Although the shoots did not have an increased iron concentration, it is highly likely that there was an overall increase in total iron since the plants were larger than the control. It is impossible to determine if this assumed iron increase was significant or not from the data reported.

The study by Barnard et al. appeared to have the greatest effect on the plants at the highest lime level, while Pilanali and Kaplan (2003) found that humic acids were less effective as the soil became more calcareous. Two major differences between the studies may help explain this contradiction: 1. one was done in quartz sand and the other was in soil, 2. the improved results were seen with a fulvic acid while the decreasing results were seen using a humic acid.

DEVELOPMENT OF PRECISION IRON STRESS USING FERRIHYDRITE COATED SAND

To determine the effect of humic and fulvic acids on plant growth, a method needed to be developed that would allow for a controlled iron stress in the plants. To resemble field conditions, ferrihydrite was chosen as the iron source.

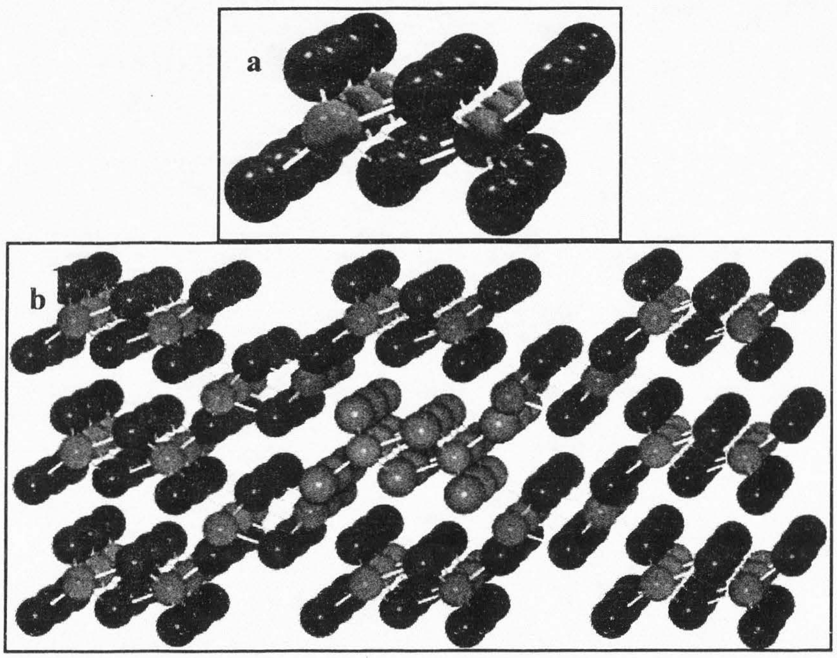

FIGURE 4. Ferrihydrite, name for both $[FeO(OH)]_8[FeO(H_2PO_4)]$ and FeO(OH). 3a) the unit cell (repeating unit; Iron (III) is brown, and oxygen (II) is red. Hydrogen is not shown). 3b) crystal-lattice structure of ferrihydrite. The gray portion shows one repeating unit (Traverso, 2004).

Ferrihydrite is a naturally occurring common iron hydroxide also known as hydrous ferric oxide, amorphous ferric hydroxide and amorphous iron oxyhydroxide. Ferrihydrite consists of many spherical particles of diameters from 1-10 nm, that aggregate together to form a loose crystalline type structure with little to no order (Dzombak and Morel, 1990) (Figure 4).

Ferrihydrite forms on soil surfaces where Fe^{2+} in water can be rapidly oxidized. A significant amount of organic matter (or other constituents that inhibit crystal formation) is associated with the formation of ferrihydrite (Bigham et al., 2002). Ferrihydrite is metastable under most natural conditions, and should remain in its current state until environmental changes occur causing it to convert to a more stable state. Ferrihydrite is a common iron oxide found in soil environments.

Iron oxides are less soluble under aerobic conditions, or when the pH of the solution is greater than 4. Ferrihydrite is the most soluble of the iron oxides; pK_{so} around 37 (Bigham et al., 2002). The surface area of Ferrihydrite is higher than most other iron oxides and ranges from 100 to 700 m^2 g^{-1}. Due to its high surface area and prevalence in soils, ferrihydrite is one of the largest sinks for nutrients and toxic elements. In addition, iron oxides and hydroxides in general absorb appreciable amounts of organic matter, the larger the organic compound the more likely it is to bind (Jardine et al., 1989), meaning that humic acids should bind to ferrihydrite more readily than fulvic acids. If the humic substances bind quickly to the ferrihydrite they should cover its surfaces and keep P from binding to the ferrihydrite and being removed from solution.

Sand was coated with ferrihydrite to study the effects of humic substances on plant uptake. The presence of ferrihydrite in a sand culture should produce a system that is representative of a field environment

MATERIALS AND METHODS

Coating sand with ferrihydrite

The procedure of coating sand with iron is:

1. Place 100 g of quartz sand into a container, add 8.08 g $Fe(NO_3)_3 \cdot 9H_2O$ (20 mmol Fe) and 50 ml of DI water to the container. Stir the sand suspension until the $Fe(NO_3)_3 \cdot 9H_2O$ dissolves.
2. Add 12 ml of 5 M NaOH to the container and stir the mixture thoroughly. Adjust the pH of the suspension to 7.5 by dropwise addition of 5 M NaOH or 6 M HCl as needed.
3. Decant off excess liquid and spread sand out on a countertop. Allow Sand to dry overnight (can use a fan to blow over the top of sand). Rewet with DI water, mix and dry the sand again.
4. Transfer the sand to a 200-μM stainless steel sieve and thoroughly rinse the sand with DI water until it is free of soluble salts and discrete oxide particles.
5. Dry the ferihydrite-coated sand.

Through preliminary work using a colorimetric iron test and DI water as an extractent it was discovered that ferrihydrite-coated sand had more than enough available iron for plants to thrive (Table 1).

Most excess iron was removed by washing the ferrihydrite-coated sand using $Ca(OH)_2$, through an exchange reaction (Figure 5). The calcium acts as a competitive cation and replaces the iron off the amorphous semi-crystalline Ferrihydrite, while the hydroxide acts to buffer the solution. This maintains a high pH keeping the amorphous iron from dissolving.

TABLE 1. Iron on ferrihydrite coated sand compared to iron needs for healthy plant growth.

Sample	Extractent	Extract [Fe] (mg/L)	Sand [Fe] (mg/kg)
Fe-Coated	DI H_2O	>5.00	>100.00
Typical plant			0.04

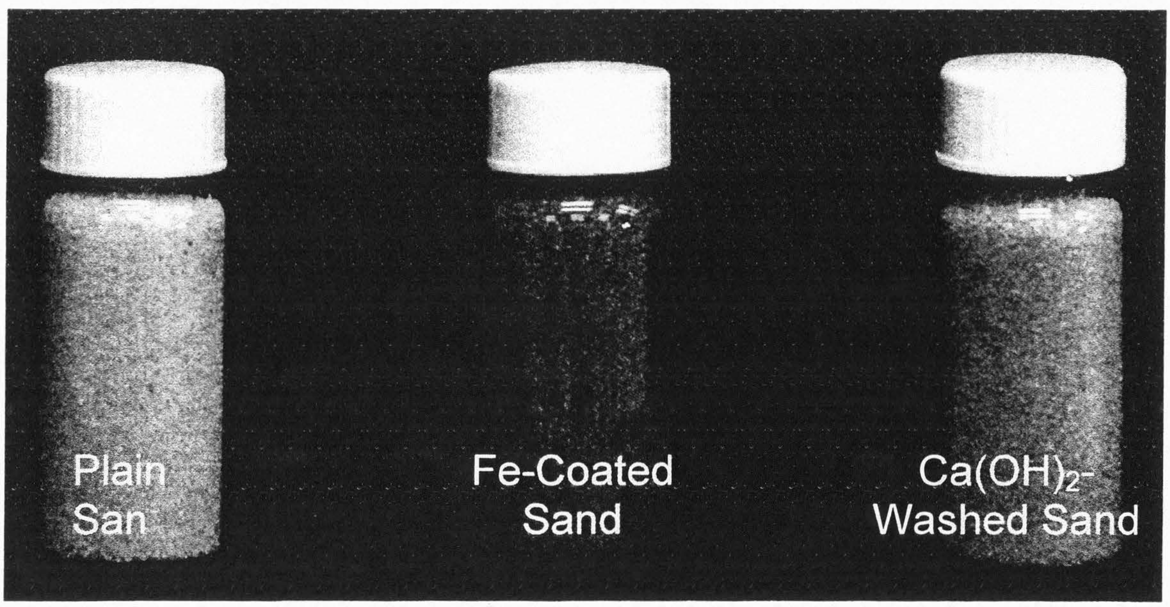

FIGURE 5. Appearance of plain Ottawa sand, Fe-coated sand, and Fe-coated $Ca(OH)_2$ washed sand. The red color is from ferrihydrite.

The $Ca(OH)_2$ washing procedure:

1. Weigh out 100 g of Fe-coated sand in a 2-L Nalgene beaker.
2. Measure out a volume of $Ca(OH)_2$ (0.02 M $Ca(OH)_2$; 1.5 g per l) equal to 10x the mass of sand and add it to the beaker.

3. Place the beaker on a stir plate, add a stir bar, and stir aggressively for one hour. Stirring for longer/shorter time periods will result in less efficient removal of iron.
4. Decant the $Ca(OH)_2$ and aggressively rinse the sand and stir bar at least three times using DI water. Be sure to wash off the ring of $Ca(OH)_2$ that formed on the inside of the beaker during stirring.
5. Add DI water to the beaker of rinsed sand. Add the same volume of DI water as the volume of $Ca(OH)_2$ added in step 2. Stir aggressively for one hour.
6. After on hour decant the DI water and aggressively rinse the sand at least three times using DI water.
7. Allow sand to air dry.

A colorimetric tests on the DI extractable iron after the $Ca(OH)_2$ washing showed that there was still plenty of iron available for plant growth (Table 2).

TABLE 2. Iron extracted from ferrihydrite coated sand after washing with $Ca(OH)_2$.

Sample	**Extractent**	**Extract [Fe] (mg/L)**	**Sand [Fe] (mg/kg)**
Fe-Coated/$Ca(OH)_2$	DI H_2O	0.30	**0.75**
Fe-Coated/$Ca(OH)_2$	DI H_2O	0.30	**0.75**
Fe-Coated/$Ca(OH)_2$	DI H_2O	0.47	**2.35**
Fe-Coated/$Ca(OH)_2$	DI H_2O	0.72	**1.80**
Fe-Coated/$Ca(OH)_2$	DI H_2O	0.22	**1.10**
Fe-Coated/$Ca(OH)_2$	DI H_2O	0.64	**1.60**
Fe-Coated/$Ca(OH)_2$	DI H_2O	0.19	**0.95**

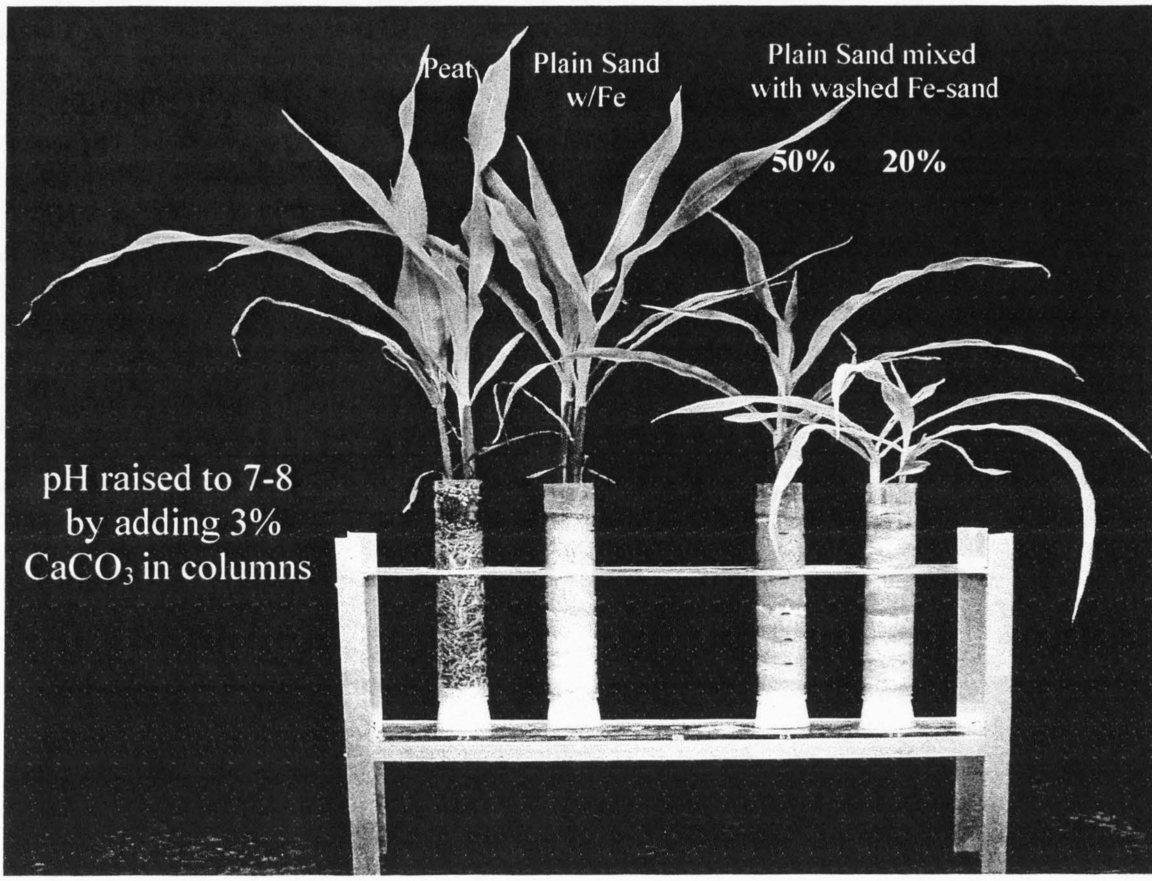

FIGURE 6. Picture of preliminary sand columns, showing peat-perlite control, a column with ferrihydrite-coated sand and two levels of plain sand mixed with ferrihydrite-coated sand. Yellowing of plants is iron chlorosis, this is associated with decreased plant size.

To produce an appropriate amount of iron stress in the plants, Ca washed Fe-coated sand would need to be mixed with normal non-Fe-coated sand. A preliminary study to find the best mixing ratio is shown in Figure 6. The results of this study suggest that the desired level of iron stress can be obtained by mixing regular sand with the Ca washed iron-coated sand at a 50-50 ratio.

Experiment 1

Maize plants (cv. DK-641) were grown in 3.5 cm diameter glass columns, cut to a length of 22 cm with a rubber stopper in the bottom. The stoppers were drilled and fitted with a 0.5 cm diameter glass tube. The total length of the stopper and the attached glass tube was 10 cm. The top 9.5 cm of this tube was packed with glass wool, the glass wool was also coiled in the bottom of the column. The glass tube was covered with black electrical tape to reduce algal growth. The column was packed with 16 cm of medium Ottawa sand (from VWR), mixed with Fe-coated (Ferrihydrite), $Ca(OH)_2$ washed medium Ottawa sand for a 70/30 mass/mass ratio. This ratio was used instead of 50/50 because our preliminary experiment did not have ideal root growth, and it was assumed that with better root growth, the plants would be able to access more ferrihydrite. Better root growth would result in plants with little or no iron stress so the ratio was changed. The columns were wrapped in alluminium foil to reduce algal growth during the experiment.

There were three replicates for each treatment and two replicates for each control. This study was conducted in a greenhouse environment with supplemental high pressure sodium lighting at 300 μmol m^{-2} s^{-1}. They were grown with a day/night cycle of 16/8 hours. The temperature in the greenhouse was 25 ^{o}C during the day and 20 ^{o}C at night. Plants were germinated in the sand columns. The humic acid treatments were applied with the water at 3.7 μl/liter (2.5 acre feet/season). The treatments were:

VK

VK-1

VK-2

F-6000

The controls were:

No organic product

Aldrich HA at 0.05 g/column (324 mg/liter)

Aldrich HA at 1.0 g/column (6.49 g/liter)

Aldrich HA at 13.0 g/column (84.4 g/liter)

The Aldrich controls (from Aldrich Chemical Company) are a series of treatments to identify the best concentration of humic substance to use in future work. The plants were watered once a day during early growth stages and twice a day thereafter. All plants were watered with the nutrient solution in Table 3. These nutrient levels represent double the standard concentration in nutrient solution. This was done so the plants would only experience iron stress.

TABLE 3. Nutrient solution used in sand columns.

Salt	ml of stock/100 L	Final Concentration
$Ca(NO_3)_2$	400	4 mM
$K(NO_3)$	300	6 mM
KH_2PO_4	100	0.5 mM
$MgSO_4$	50	0.5 mM
K_2SiO_3	100	0.1 mM
$MnCl_2$	20	12 uM
$ZnCl_2$	30	6 uM
H_3BO_3	5	2 uM
$CuCl_2$	20	4 uM
Na_2MoO_4	10	0.1 uM

pH was adjusted to 5.6 using 1 M HNO_3 as needed.

Chlorophyll content was measured weekly using a Minolta SPAD-502 meter. The plants were harvested on the 27th day of the experiment. The plants were visually scored by two persons on their overall yellowing, and purpling. Fresh and dry weights were measured. The roots were washed and visually scored by two persons. All statistical analyses were done by an ANOVA test using SAS software (Version 9.0).

Experiment 2

This experiment was run the same as experiment one with the following changes. The columns were packed with 16 cm of coarse golf course sand. This sand was used because of its angularity, compared to Ottawa sand (Figure 7).

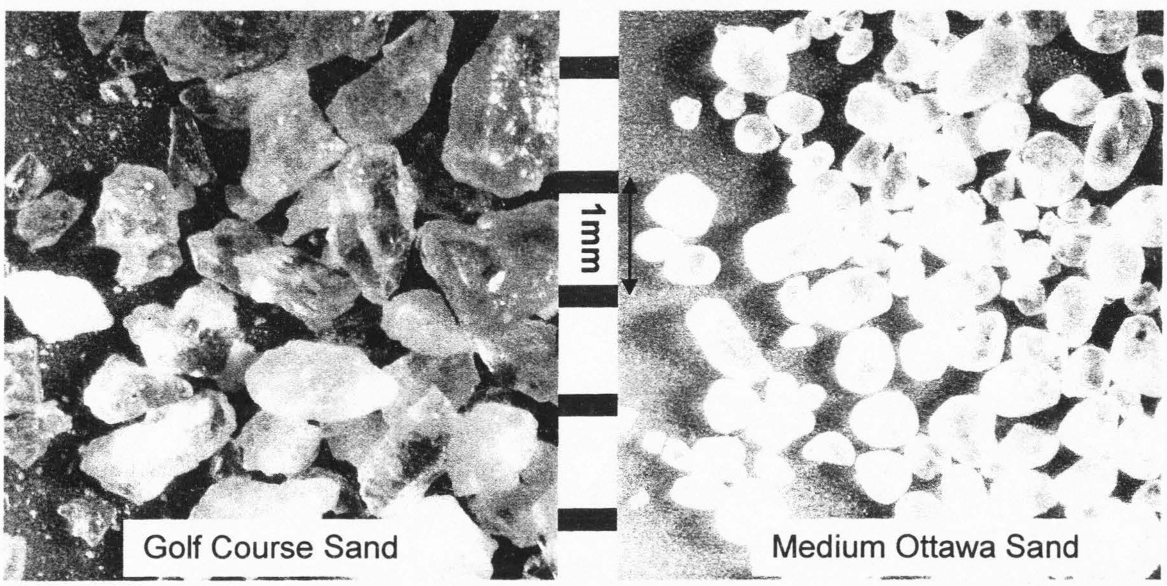

FIGURE 7. Pictures taken through a microscope of golf course (angular) and Ottawa (round) sands, with a 1-mm scale.

The more angular sand increases porosity and improves water and air content in the root zone. This should result in improved root growth over the previous experiment. The golf course sand was mixed with a iron coated (Ferrihydrite) $Ca(OH)_2$ washed Ottawa sand mixture (80 fine, 20 large) to get a 20 % ferrihydrite (mass/mass) coated sand mixture.

The sand was mixed with 3% $CaCO_3$ (v/v) to buffer pH levels between 7.3 - 8.0, at this pH range iron is not readily available. The $CaCO_3$ was mixed with 0.5% $CaCl_2$ to initiate the buffering reaction. The buffering occurs faster with some excess Ca to start it. The high pH will reduce solubilities of most nutrients. There were three replicates for each treatment. The humic acid treatments were applied with the water at 50 µl/liter. The treatments were:

VK

VK-1

VK-2

F-6000

The controls were:

No organic product (without Fe coated sand; with $CaCO_3$)

No organic product (with Fe coated sand; with $CaCO_3$)

No organic product (with Fe coated sand; without $CaCO_3$)

Aldrich HA at 0.05 g/column (324 mg/liter + 2.5 mg/liter)

Aldrich HA at 1.0 g/column (6.49 g/liter + 50 mg/liter)

Aldrich HA at 13.0 g/column (84.4 g/liter + 1000 mg/liter)

The controls represent two series of treatments. These are a test of the effectivness of our procedure. The series are listed in order from the treatments that should do the worst to the ones that should perform best. The "with Fe with $CaCO_3$" treatment was the control used for comparison among treatments. The Aldrich HA controls were mixed thoughout the sand column at the beginning of the study and were watered with additional HA mixed in with the nutrient solution (as noted in the list of controls). The plants were watered sparingly throughout germination and emergence, then once a day through most of the experiment. When the plants became large, watering was increased to twice a day.

Chlorophyll content was measured weekly using an Opti-Sciences CCM-200 chlorophyll content meter, on the newest emerged leaves. The Opti-Sciences meter has a larger mearuring area than the Manolta meter giving better measurements. When possible two leaves were measured starting with the third leaf. The plants were harvested on the 26th day of the experiment, and fresh weights taken. The roots were washed and inspected for treatment effects.

RESULTS

Experiment 1

There was no significant difference among the chlorophyll contents between treatments throughout the experiment, or in the visual analysis of the plants. There were significant differences in root growth (Table 4). The Aldrich treatments were applied at rates approximately 100 to 10,000 times higher than the other treatments.

The differences in root growth did not come out as expected; Aldrich at 0.05 g had more roots than Aldrich at 1.0 g. The purple color (Figure 8) and the Aldrich series being out of order suggested non-ideal plant growth.

TABLE 4. Average root growth with statistical analysis.

Treatment	Mean	
Aldrich 0.05	4.25	a
Aldrich 13	4.25	a
VK-1	3.75	ab
Aldrich 1	3.50	ab
Control	3.13	bc
VK	2.63	c
F-6000	2.33	c
VK-2	1.42	d
F Value	13.92	
Pr > F	<.0001	
Coeff Var	19.15	

* Treatments with different letters are significantly different at the p = 0.05 level.

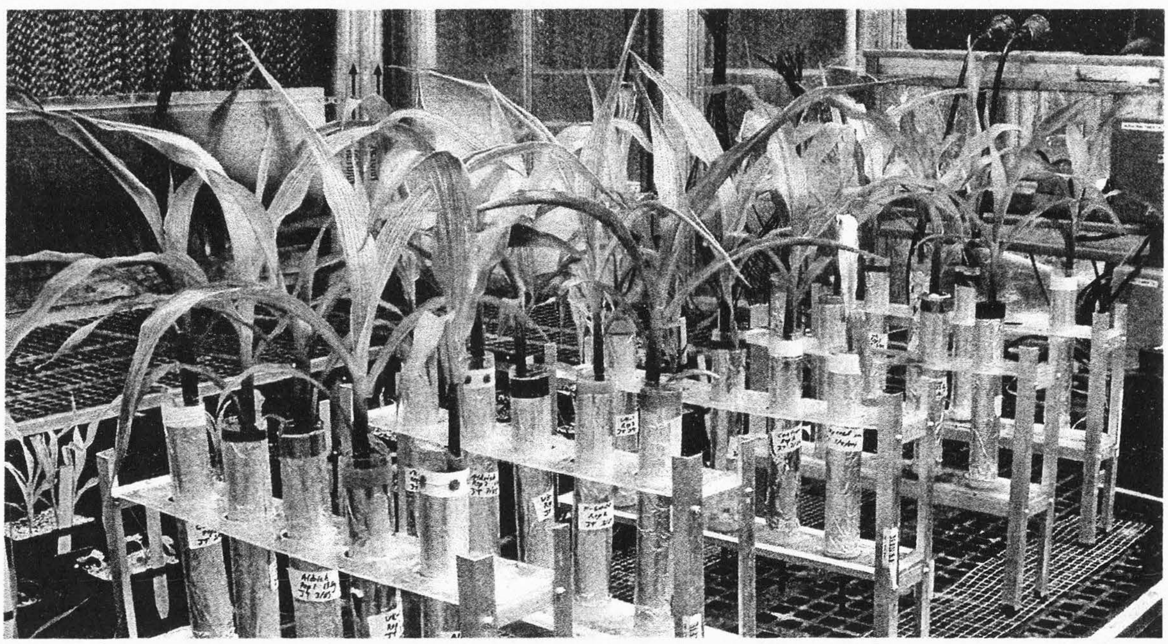

FIGURE 8. Maize plants growing in sand columns. Columns were marked with colored tape to facilitate treatment application. Yellow coloring is iron chlorosis.

Experiment 2

The first chlorophyll content measurements were taken on the 14th day of the experiment. Aldrich HA at 13 g and at 1 g rates were both significantly better than the control (Table 5). The "without Fe with $CaCO_3$" treatment had more than average chlorophyll content. Because there was no available iron in this treatment it was expected to be the most chlorotic. The plants most likely were still using iron stored in the seeds at this point. Chlorophyll content values less than ten are severely chlorotic.

TABLE 5. Average chlorophyll content of the third leaf on the 14th day after planting; with statistical analysis.

Leaf 3 Day 14		
Aldrich 13g	17.1	a
Aldrich 1g	9.3	b
Without Fe with $CaCO_3$	7.4	bc
F-6000	6.7	bcd
With Fe without $CaCO_3$	6.3	bcd
Aldrich 0.05g	5.7	bcd
With Fe with $CaCO_3$	4.7	cd
VK-2	4.3	cd
VK-1	3.7	cd
VK	3.4	d
F Value	9.32	
Pr > F	<.0001	
Coeff Var	30.6	

* Treatments with different letters are significantly different at the $p = 0.05$ level.

The chlorophyll readings on the 17th day show that Aldrich at 13 g had statistically more chlorophyll than all other treatments (Table 6). Aldrich with 1 g had statistically more chlorophyll than the control as well. There was a small increase in chlorophyll content in general over these few days.

TABLE 6. Average chlorophyll content of the third leaf on the 17th day after planting; with statistical analysis.

Leaf 3 Day 17		
Aldrich 13g	27.8	a
With Fe without $CaCO_3$	12.0	b
Aldrich 1g	11.1	bc
Without Fe with $CaCO_3$	8.6	bcd
Aldrich 0.05g	6.7	bcd
F-6000	5.9	bcd
With Fe with $CaCO_3$	5.9	bcd
VK	4.3	cd
VK-1	3.4	d
VK-2	3.3	d
F Value	8.59	
Pr > F	<.0001	
Coeff Var	45.0	

* Treatments with different letters are significantly different at the p = 0.05 level.

On the 19th day all the plants were large enough to begin measuring the fourth leaf (Table 7). The third leaf data showed little change from the last week, except the Aldrich 13 g was now the only significant treatment. Aldrich at 13 g had significantly more chlorophyll than all other treatments in the fourth leaf and the "with Fe without $CaCO_3$" treatment had statistically more chlorophyll than the control (Table 7).

This situation continued through the next readings (Table 8), generally the treatments continued to get greener over time.

TABLE 7. Average chlorophyll content of the third and fourth leaves on the 19th day with statistical analysis.

Leaf 3 Day 19			Leaf 4 Day 19		
Aldrich 13g	28.9	a	Aldrich 13g	16.3	a
With Fe without $CaCO_3$	12.3	b	With Fe without $CaCO_3$	8.6	b
Aldrich 1g	11.1	bc	Aldrich 1g	4.2	c
Without Fe with $CaCO_3$	8.1	bc	VK	3.2	c
Aldrich 0.05g	7.5	bc	VK-1	3.1	c
With Fe with $CaCO_3$	6.4	bc	With Fe with $CaCO_3$	3.1	c
VK-1	5.6	bc	Without Fe with $CaCO_3$	3.1	c
VK	4.2	c	F-6000	2.7	c
VK-2	4.1	c	Aldrich 0.05g	2.5	c
F-6000	3.9	c	VK-2	2.1	c
F Value	7.49		**F Value**	19.26	
Pr > F	0.0001		**Pr > F**	<.0001	
Coeff Var	47.8		**Coeff Var**	33.6	

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE 8. Average chlorophyll content of the third and fourth leaves on the 21st day with statistical analysis.

Leaf 3 Day 21			Leaf 4 Day 21		
Aldrich 13g	33.5	a	Aldrich 13g	28.5	a
Aldrich 1g	16.6	b	With Fe without $CaCO_3$	11.7	b
With Fe without $CaCO_3$	14.2	bc	Aldrich 1g	5.4	c
Without Fe with $CaCO_3$	10.1	bcd	VK	4.7	c
Aldrich 0.05g	8.9	bcd	Without Fe with $CaCO_3$	3.3	c
With Fe with $CaCO_3$	5.8	cd	VK-2	3.2	c
VK-2	4.8	d	VK-1	3.0	c
F-6000	4.7	d	With Fe with $CaCO_3$	3.0	c
VK	4.5	d	F-6000	2.8	c
VK-1	4.1	d	Aldrich 0.05g	2.5	c
F Value	8.24		**F Value**	46.5	
Pr > F	<.0001		**Pr > F**	<.0001	
Coeff Var	48.3		**Coeff Var**	29.0	

* Treatments with different letters are significantly different at the p = 0.05 level.

The last chlorophyll measurements taken on the 24th day used the fourth and fifth leaves. Both leaves showed Aldrich 13 g treatment having the largest plants with the most chlorophyll followed by the "with Fe without $CaCO_3$" treatment (Table 9). Both treatments were statistically better than the control but were also statistically different than each other. As hypothesized the Aldrich HA treatments ended with 13 g being the best and 1 g and 0.05 g following in that order. The iron sand controls also line up as expected; with the treatment without $CaCO_3$ having the most chlorophyll followed by the iron with $CaCO_3$ and last was the treatment without iron. The plants grew well in this experiment and did not have purpling like the first experiment (Figure 9).

TABLE 9. Average chlorophyll content of the fourth and fifth leaves on the 24th day with statistical analysis.

Leaf 4 Day 24			**Leaf 5 Day 24**		
Aldrich 13g	29.1	a	Aldrich 13g	19.3	a
With Fe without $CaCO_3$	12.6	b	With Fe without $CaCO_3$	7.2	b
Aldrich 1g	5.7	c	Aldrich 1g	3.1	c
VK	4.9	c	VK	3.0	c
Aldrich 0.05g	3.2	c	With Fe with $CaCO_3$	2.5	c
With Fe with $CaCO_3$	3.2	c	Without Fe with $CaCO_3$	1.9	c
VK-2	3.1	c	F-6000	1.8	c
Without Fe with $CaCO_3$	2.9	c	Aldrich 0.05g	1.7	c
F-6000	2.8	c	VK-2	1.6	c
VK-1	2.5	c	VK-1	1.6	c
F Value	37.98		**F Value**	36.01	
Pr > F	<.0001		**Pr > F**	<.0001	
Coeff Var	32.1		**Coeff Var**	35.0	

* Treatments with different letters are significantly different at the $p = 0.05$ level.

FIGURE 9. Maize plants grown in sand columns. Yellow coloring is iron chlorosis. Columns were marked with colored tape to facilitate treatment application.

TABLE 10. Average fresh mass with statistical analysis.

Fresh Mass Day 26		
With Fe without $CaCO_3$	12.8	a
Aldrich 13g	12.1	ab
Aldrich 1g	10.0	bc
With Fe with $CaCO_3$	8.7	cd
Aldrich 0.05g	8.7	cd
Without Fe with $CaCO_3$	8.5	cd
F-6000	8.4	cd
VK	7.8	cd
VK-1	7.4	cd
VK-2	7.0	d
F Value	4.92	
Pr > F	0.0017	
Coeff Var	15.9	

* Treatments with different letters are significantly different at the p = 0.05 level.

The harvest data shows Aldrich 13 g and "with Fe without $CaCO_3$" treatments were statistically larger than the other treatments (Table 10). Once again the two series ended up in their expected orders. The FA treatments besides Aldrich were the smallest plants in this study but were not statistically so.

DISCUSSION

The poor root growth and the purple color in the plants particularly near the stems of experiment one suggested less than ideal plant growth. It is doubtful this was caused by a nutrient problem considering the abundance of nutrients supplied. The physical properties in the root zone were most likely the cause of poor plant growth. In reviewing the methods, it was decided that the use of an angular sand (golf course sand) would improve plant growth conditions in experiment two.

The root growth in experiment two was very poor except for the Aldrich 13 g treatment, which had decent, but not great root growth. Overall, better root growth was expected in this experiment because of the switch from Ottawa sand to the more angular golf coarse sand. Changing sands should have allowed for a more loosely packed column and increased pore space, therefore allowing increased root growth in this experiment, however, the opposite was observed. The same result was observed in an ancillary study. A portion of this decreased root growth was the golf course sand is larger then the Ottawa sand (Figure 6) and would have resulted in a decrease in porosity. It is also possible that the slightly different watering technique during germination had some effect on root growth, but presumably this was small. Even so, it is suggested that a more routine watering method be used in future work.

In several side studies done throughout the time of these experiments, good root growth was observed using this system both with Ottawa sand and golf course sand. Why this was not observed in these experiments is puzzling, but one study done after the experiments showed that, the larger the columns the better the overall root growth. In future studies it would be worth using larger PVC columns.

In experiment one, the Aldrich HA generally tended to improve root growth where the other humic substances did not affect root growth. The Aldrich treatments were applied at approximately 100 to 10,000 times higher concentrations than VK, VK-1, VK-2 and F-6000 treatments. Humic substances can improve root growth but at much higher rates than the VK, VK-1, VK-2 and F-6000 treatments were applied. These findings are in agreement with information mentioned in chapter one that the largest affects of humic substances is usually seen on root growth. In future studies the rate of application should be increased in an attempt to improve treatment effects.

In experiment two Aldrich 13 g treatment had the most chlorophyll in the leaves, the best root growth and some of the largest plants in the study, while all other treatments were significantly less in these growth areas. The largest difference between this and the other treatments was the level of HA application. It was applied at a rate at least 5000 times higher than the F-6000, VK, VK-1 and VK-2. The level used for the fulvic acids in this experiment was 50 μl/liter, 50 μl/liter as noted in chapter one, is typically the bottom end of the range where differences are seen in hydroponics. Concentration around 300 μl/liter, the high end of the typical hydroponics range, might increase effects to noticeable levels. Increasing the concentration of the non-Aldrich humic substances should result in noticeable differences and the best HA/FA for improving iron chlorosis could be identified. The other major differences are, the non-Aldrich products are liquid fulvic acids and not dry granular humic acids. Additionally Aldrich humic acid could be analyzed for nutrient impurities to make sure that the observed effects on iron uptake are a result of iron complexation and not easy iron already attached to the humic acid.

The control series in experiment two came out as expected on chlorophyll content and plant mass with the order of the iron coated sand control series being; "with Fe without $CaCO_3$", "with Fe with $CaCO_3$," and the "without Fe with $CaCO_3$." This series of treatments were included as a check on the effectiveness of our experimental system. The fourth leaf's data was the first to show this ordering. Implying that there is adequate iron stored in the seed to get the plant through the first three leaves. Suggesting the fourth and fifth leaves most likely represent treatment effects.

The "with Fe without $CaCO_3$" treatment was better than all other treatments except Aldrich 13 g in chlorophyll content. The "with Fe without $CaCO_3$" treatment should have had adequate iron because it had no root zone pH control. Plants that had no pH control could release siderophores (organic acid released by plant roots) to lower the pH of the rhizosphere and chelate iron making it readily available. The fresh mass closely follows the chlorophyll content data except that the top two treatments are reversed.

This experiment showed that Aldrich HA 13 g treatment had much greener leaves than the rest of the treatments. Because of the very high rates of HA application it was expected that this treatment would do well. In addition, this same product applied at 1 g also tended to do well but not significantly so.

Interestingly, the Aldrich humic acid in this study has not shown bad results at high pH levels like Pilanali and Kaplan (2003) observed. This may mean that their finding relates more to how humic acids react in soils and not how humic acids react in general.

Linehan (1978) found that humates react very slowly with iron hydroxides. This means that using ferrihydrite is a good choice as an iron source because of its high surface area and reactivity compared to other iron oxides and hydroxides. Suggested future work using goethite may not be as beneficial as once thought.

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CHAPTER 3

THE EFFECT OF DRY GRANULAR HUMIC ACID PRODUCTS ON TOMATO GROWTH AND YIELD

ABSTRACT

Little research has been done on coal derived humic substances in dry granular form on soil systems in long term studies. This study examined the effects of dry granular coal derived humic substances effects on tomato plant growth and yield grown in soil columns. A total of 11 commercially-available dry granular humic acid products were evaluated for their effects on tomato plant growth and yield in two experiments. Only one treatment DGX FeZnMn Blend from Horizon Ag Products (Modesto, CA), at a rate of 44.8 kg/ha (40 lbs/acre) produced any beneficial effects on plant growth. The Blend treatment improved root growth, but did not improve shoot growth or fruit yield. Most dry granular products do not improve plant growth and yield when applied at the low rates used in this study.

INTRODUCTION

Few researchers have studied leonardite-derived compounds and their effects on plant growth. David et al. (1994) studied a commercially available leonardite-extracted HA treatment on tomato plants in a 22 day study. They found that the leonardite treatment significantly increased the dry mass of tomato roots when it was supplied at 1280 mg/liter in hydroponics solution, but had no effect when it was supplied at half or double that concentration.

In another hydroponic system Adani et al. (1998) looked at plant response to two commercially available humic acids, which were derived from peat and leonardite. Both products were applied at 20 and 50 mg/liter. The leonardite product improved shoot and root fresh and dry masses at 50 mg/liter, but only shoot dry mass at 20 mg/liter. In addition they analyzed both roots and shoots for N, K, Ca, Mg, P, Fe, and Cu. In the shoots, the leonardite product improved P concentration at 50 mg/liter. In the roots, it increased P and Fe at 50 mg/liter and Fe and 20 mg/liter. The peat derived product had a larger effect on nutrient uptake than the leonardite product. Barnard et al. (1992) also found increased iron uptake in maize plants grown in quartz sand using coal derived product.

Cooper et al. (1998) used a dry granular product in quartz sand. Growing creeping bentgrass they observed increased root mass and an overall increase in root length. In addition they noticed an increase in P and Mg.

The researchers above found beneficial results using leonardite derived humic materials, but all their work was done in hydroponics or sand, few projects have used soil. Fagbenro and Agboola (1993) did use soil although they did not use a coal-derived product. They studied teak tree seedlings in two soils over a four-month period. A soil derived humic acid at 50, 500, 1000 mg/kg rates was used and they found increased plant height in the oxisol soil at all rates, but tree diameter was only increased at the highest rate. In the alfisol the only increase was on plant height at the highest concentration. Dry matter yield and root/shoot ratio were all increased except for the alfisol at 500 mg/kg. There was an increase seen in N, P, K, Ca, Zn, and Fe in the plants grown in the alfisol soil but the treatment level of these differences varied. The oxisol had increases in K

(1000 mg/kg), Ca (500 and 1000), and Fe (500 mg/kg rate) in the plants. The humic acid appears to have a larger effect on plant grown in the less fertile oxisol soil.

One study that used both soil as the growth media and a dry granular product was Pilanali and Kaplan (2003). In this study strawberry plants were grown in a calcareous soil over two years, they found that the humic product applied at 100, 200, 300, and 400 kg/ha had no beneficial effects on plant growth or nutrient uptake. The length of this experiment is unusual, most humic substances studies are less than 30 days.

MATERIAL AND METHODS

Experiment 1

Tomato plants (cv. Micro-Tom) were grown in 7.6 cm (3") diameter columns made of PVC pipe cut to a length of 46 cm (18") with a PVC cap on one end (Figures 10 and 11). A drain hole in the middle of the cap was covered with 16-mesh screen to keep soil in the column. The columns were prepared by mixing a sandy loam soil from eastern Idaho with an equal amount of sand to get a 50/50 soil/sand mixture by volume. The soil characteristics were:

Texture-	Sandy Loam	
pH	7.8	
EC	1.5	
Organic Matter (%)	2.4	Walkley-Black
$CaCO_3$ (%)	1.4	
P (mg/kg)	4.8	Olsen $NaHCO_3$ test
K (mg/kg)	209	Olsen $NaHCO_3$ test

FIGURE 10. Tomatoes grown in soil columns (picture taken on the 85th day of the experiment).

An 11-52-0 fertilizer at a rate of 224 kg/ha (200 lbs. per acre; or 0.112 g per 50.3 cm^2 column surface area) was mixed with the dry granular humic acid (DG HA) products. Then these mixtures were incorporated into the top 8.9 cm (3.5 inches) of the columns by scooping the soil out of the columns and hand mixing.

The humic acid products and their rate of application were:

Agri-Plus	44.8 kg/ha	(40 lbs/acre)
DGX MS-A	44.8 kg/ha	(40 lbs/acre)
DGX MS-JA	44.8 kg/ha	(40 lbs/acre)
DGX MS-JB	44.8 kg/ha	(40 lbs/acre)
High Grade 70%	8.41 kg/ha	(7.5 lbs/acre)
Luscar Spray Dry	3.36 kg/ha	(3 lbs/acre)
Morningstar Micronized	44.8 kg/ha	(40 lbs/acre)
North Dakota Leonardite	44.8 kg/ha	(40 lbs/acre)
Quantum-H Spray Dry	3.36 kg/ha	(3 lbs/acre)

The controls were:

Aldrich chemical co. HA	44.8 kg/ha	(40 lbs/acre)
Column with fertilizer but no HA	-0-	-----
Column with no fertilizer or HA	-0-	-----

There were three replicate columns in a complete randomized design. All columns were watered with tap water throughout the study, approximately every two to three days. The columns were leached weekly to recharge column water content.

Ten to fifteen seeds were started in each column, and thinned down to one plant over the two weeks following emergence. Plants were selected for uniformity. The plants were grown in a greenhouse with supplemental light from high pressure sodium lamps. They were grown with a day/night cycle of 16/8 hours.

Harvest of fruit was done as fruit became ripe. Parameters measured were number of fruit, fresh and dry mass. Plants were harvested on the 97th day of the experiment, all remaining fruit; both ripe and unripe was harvested. Roots were judged on a scale from one to five with five being the best. All statistical analyses were done by an ANOVA test using SAS software (Version 9.0).

Experiment 2

Experiment two had the following differences. A different cultivar of Tomato (cv. Red Robin) was used. Red Robin is a slightly larger cultivar than Micro-Tom, it was hoped that this would result in stronger germination and growth. In addition Red Robin is commercially available while Micro-Tom is not.

The fertilizer in this experiment was incorporated into the column between 5-12.7 cm (2-5 inches) deep, then a layer of soil 5 cm (2 inches) thick was placed on top. This was done to minimize the chance of root burn in the young plants. The humic acid products and their rate of application were:

Agri-Plus	44.8 kg/ha	(40 lbs/acre)
DGX MS-A	44.8 kg/ha	(40 lbs/acre)
DGX MS-B	44.8 kg/ha	(40 lbs/acre)
DGX MS-JA	44.8 kg/ha	(40 lbs/acre)
High Grade 70%	8.41 kg/ha	(7.5 lbs/acre)
North Dakota Leonardite 2	44.8 kg/ha	(40 lbs/acre)
DGX FeZnMn Blend	44.8 kg/ha	(40 lbs/acre)

The controls were:

Control no HA w/ fertilizer	-0-	-----
Control no HA w/o fertilizer	-0-	-----

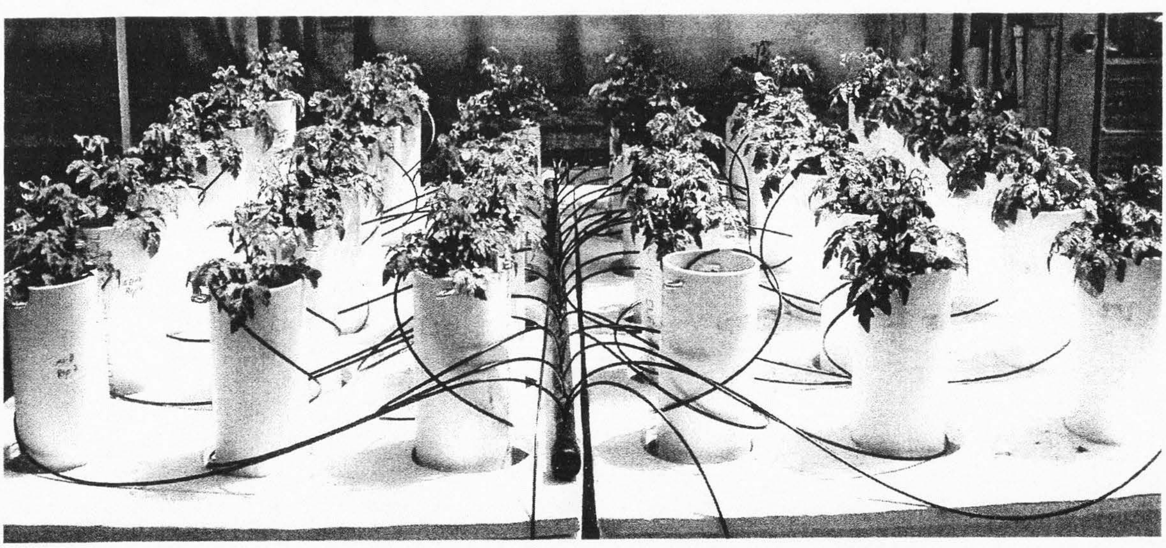


FIGURE 11. Tomatoes grown in soil columns during the dry granular experiment 2 with automated watering system.

There were four replicate columns in a complete randomized design in this experiment. An automatic water system was used, so each column would get similar amounts of water and to reduce ponding on the columns surface (Figure 11).

Digital pictures were taken of each plant each week (Figure 12). Pixel counts were measured (using Adobe Photoshop 6.0) for the plants to calculate relative growth rates. This method of measuring plant growth was described in a paper by Klassen et al. (2003). After the 48th day pictures were no longer taken because the layering of the leaves made this method less valuable.

FIGURE 12. The digital photography method and pixel counting procedure for examining early growth.

Plants were harvested on the 65th day of the experiment. Root growth was also analyzed in this experiment. Roots were judged on a scale from one to five with five being the best. They were judged on how far down the column substantial root growth had progressed, and on how well they infiltrated the entire column. Both controls received supplemental fertilization during the experiment so in the harvest analysis they were combined as one treatment.

RESULTS

Experiment 1

Root growth was similar for all plants; the roots used the entire length of the columns (Figure 13).

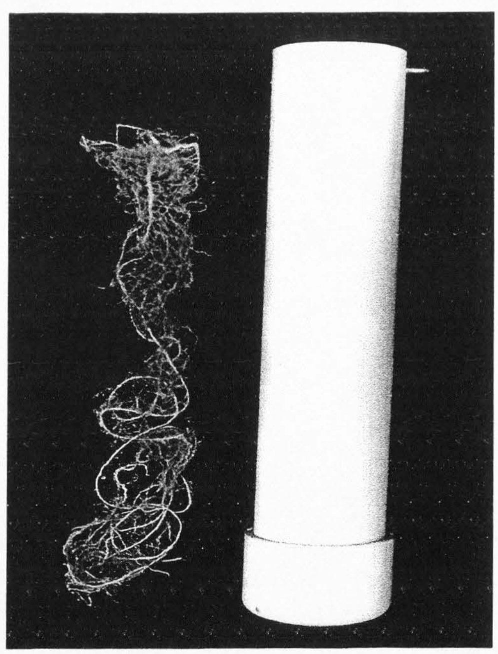


FIGURE 13. Photo of a representative plants roots and column. There was good root growth in the columns.

Red (ripe) fruit data

The treatments that produced the greatest mass of red fruit were ND leonardite, MS-JA and HG 70 %. All the treatments except for MS-JB and Micronized exceeded the controls (Figure 14A). However none of these differences were statistically significant in this study (Table 11A).

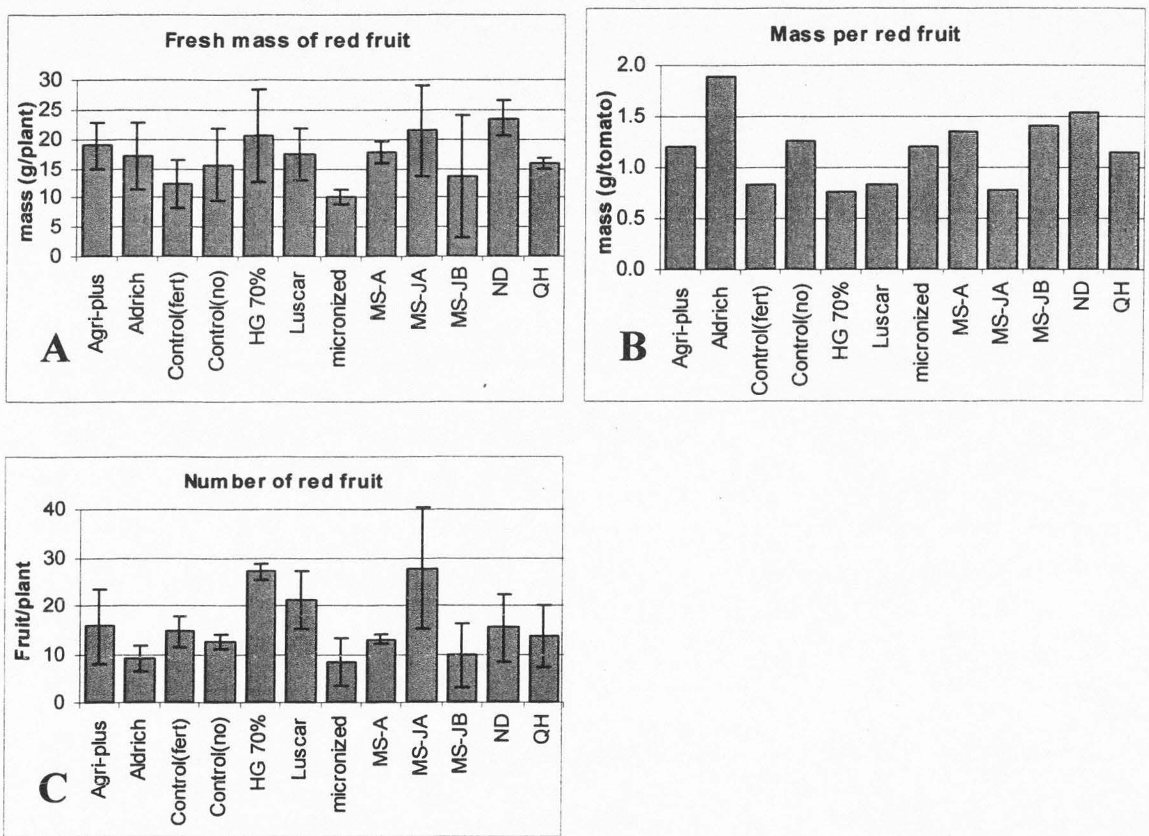


FIGURE 14. A) Fresh mass of fruit per plant. B) fresh mass of each ripe fruit. C) number of ripe fruit per plant.

Of the three treatments that produced the most red fruit mass, only the ND leonardite had a greater mass per tomato than the controls, both MS-JA and HG 70% were less than the controls. Luscar also had an average mass per fruit less than the control (Figure 14B). There was no significant treatment effect on mass per fruit (Table 11A).

Luscar, HG 70% and MS-JA produced a greater number of red fruit than the controls, and Micronized, Aldrich and MS-JB produced less fruit than the controls (Figure 14C). There were statistically significant differences in the number of fruit produced per plant, with MS-JA and HG 70% producing more than the control and all other treatments except Luscar (Table 11A; 11B).

TABLE 11. A) Statistical analysis of the ripe fruit data, B) mean separation of fruit number.

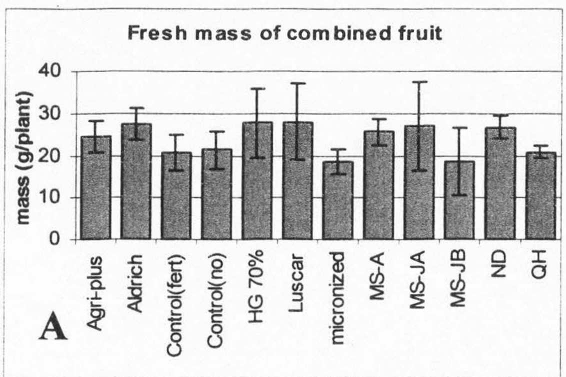
A	DF	F Value	Pr > F	Coeff Var
number of fruit	11	3.41	0.006*	38.8
fresh mass	11	1.42	0.23	32.8
mass/fruit	11	1.11	0.40	53.2
% fresh	11	0.48	0.90	7.3

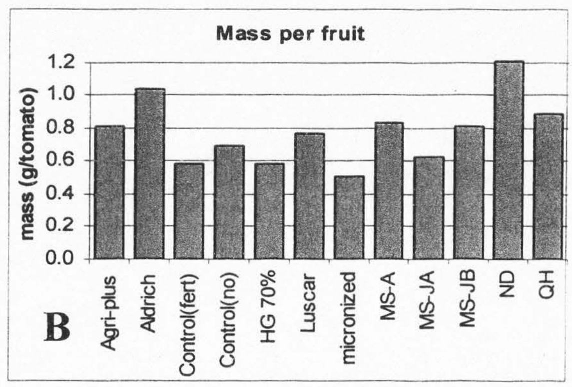
B

Treatment	Number of fruit		% of control
MS-JA	27.7	a	189
HG 70%	27.0	a	184
Luscar	21.0	ab	143
Agri-plus	15.7	bc	107
ND	15.3	bc	105
Control (w/fert)	14.7	bc	100
QH	13.7	bc	93
MS-A	13.0	bc	89
Control (w/o fert)	12.3	bc	84
MS-JB	9.7	c	66
Aldrich	9.0	c	61
Micronized	8.3	c	57
LSD	10.2		

Combined (red and green) fruit data
(Green fruit data is in Appendix C)

The combined data for the red and green tomatoes suggest that all the treatments produced more tomato mass than the controls except for Micronized, MS-JB and QH (Figure 15A), but this was not significant (Table 12A). ND leonardite and Aldrich treatments had the greatest mass per fruit (Figure 15B), while HG 70% and MS-JA treatments produced the largest number of fruit (Figure 15C). None of these differences were significant.





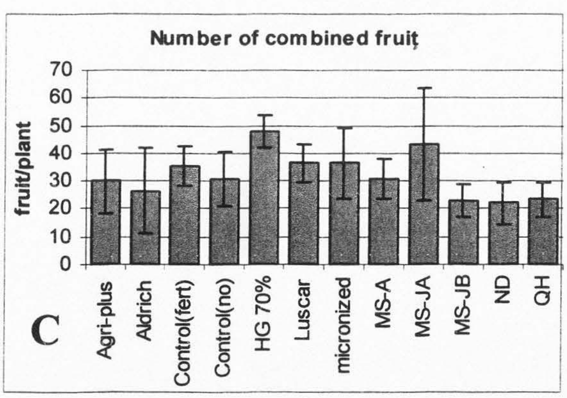


FIGURE 15. A) Fresh mass of all fruit per plant. B) fresh mass of each fruit. C) number of fruit per plant.

TABLE 12. A) Statistical analysis of the combined fruit data, B) mean separation of fruit number.

A	DF	F Value	Pr > F	Coeff Var
number of fruit	11	1.73	0.13	33.1
fresh mass	11	1.14	0.38	24.7
mass/fruit	11	1.53	0.19	41.6
% fresh	11	0.39	0.95	8.1

B

Treatment	Number of fruit	% of control
HG 70%	47.7	135
MS-JA	43.3	123
Luscar	36.3	103
Micronized	36.3	103
Control (w/fert)	35.3	100
MS-A	30.7	87
Control (w/o fert)	30.7	87
Agri-plus	30.0	85
Aldrich	26.3	75
QH	23.3	66
MS-JB	23.0	65
ND	22.0	62
	n.s.	

Results for percent red fruit by number
and the percent red fruit by mass

The percent red fruit by number showed large differences (Figure 16), with ND and MS-JA being significantly greater than the control (Tables 13A, 13B). The percent red fruit by mass did not have differences as large (Figure 17; Table 13A).

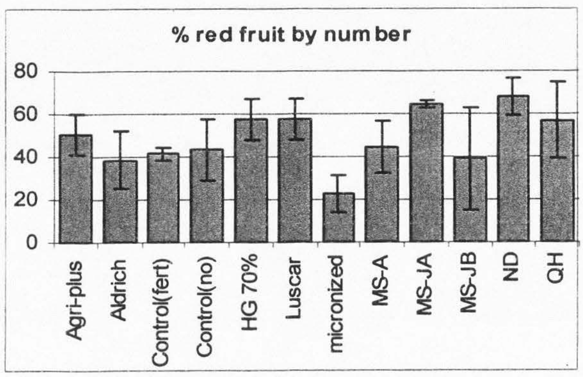


FIGURE 16. Percent of the fruit number that were red.

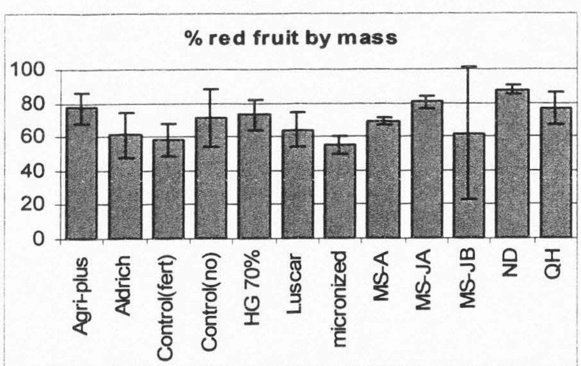


FIGURE 17. Percent of the fruit fresh mass that was red.

TABLE 13. A) Statistical analysis of the percent red fruit, B) mean separation of the percent red fruit by number.

A	DF	F Value	Pr > F	Coeff Var
% red fruit by number	11	3.2	0.008*	25.5
% red fruit by mass	11	1.37	0.25	21.0

B Treatment	% red fruit by number		% of control
ND	67.8	a	163
MS-JA	64.4	ab	155
HG 70%	57.4	abc	138
Luscar	57.2	abc	138
QH	56.9	abc	137
Agri-plus	50.4	abc	121
MS-A	44.2	bc	107
Control (w/o fert)	43.2	cd	104
Control (w/fert)	41.5	cd	100
MS-JB	38.9	cd	94
Aldrich	38.5	cd	93
Micronized	22.7	d	55
LSD	20.9		

Vegetative plant mass data

There was no significant difference in the vegetative mass of the harvested plants (Figure 18; Table 14). There were no significant differences in percent fresh mass (Tables 11A, 12A, and 14).

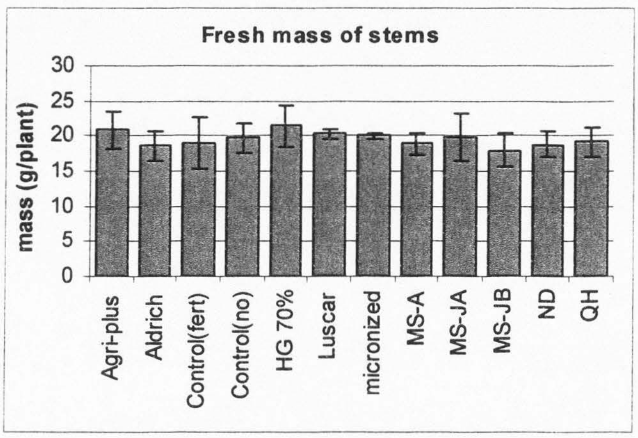


FIGURE 18. Stem mass for each treatment.

TABLE 14. Statistical analysis of the plant stems.

	DF	F Value	Pr > F	Coeff Var
fresh mass	11	0.56	0.84	12.12769
% fresh	11	0.63	0.79	4.577361

Experiment 2

One replicate from the Blend treatment had a plant with a damaged meristem; as a result the plant quit growing and was removed from the study.

There were no statistically significant differences in growth rates through the first 26 days (Figure 19, differences are none significant Table 15). During early growth, plants go through an exponential phase. It is during this stage that treatment effects should start to become evident. Figure 20 shows plant growth after the initial exponential phase start to level off. The average kilo-pixel size of the plants in each treatment through the first 48 days is shown in Table 15 and Figure 20.

TABLE 15. Kilopixel counts for each treatment for each week of the experiment. Numbers in the table represent averages of four plants. None of the differences in this table are statistically significant.

Treatment	Day 12	Day 19	Day 26	Day 33	Day 40	Day 48
Control w/ fertilizer	50.8	129	377	669	779	999
Control w/o fertilizer	47.6	115	354	671	783	1015
HG 70%	48.7	117	296	559	711	897
Blend	56.2	152	446	747	936	1170
MS-A	48.9	121	389	690	774	1037
MS-B	49.6	136	408	633	741	935
MS-JA	58.0	162	428	648	774	1009
ND	52.2	139	406	690	832	1068
Agri-plus	47.8	115	332	710	890	1138
F Value	0.63	1.29	0.82	0.84	0.94	1.43
Pr > F	0.74	0.29	0.59	0.58	0.50	0.23
Coeff Var	17.86	22.33	26.84	16.74	17.44	13.64

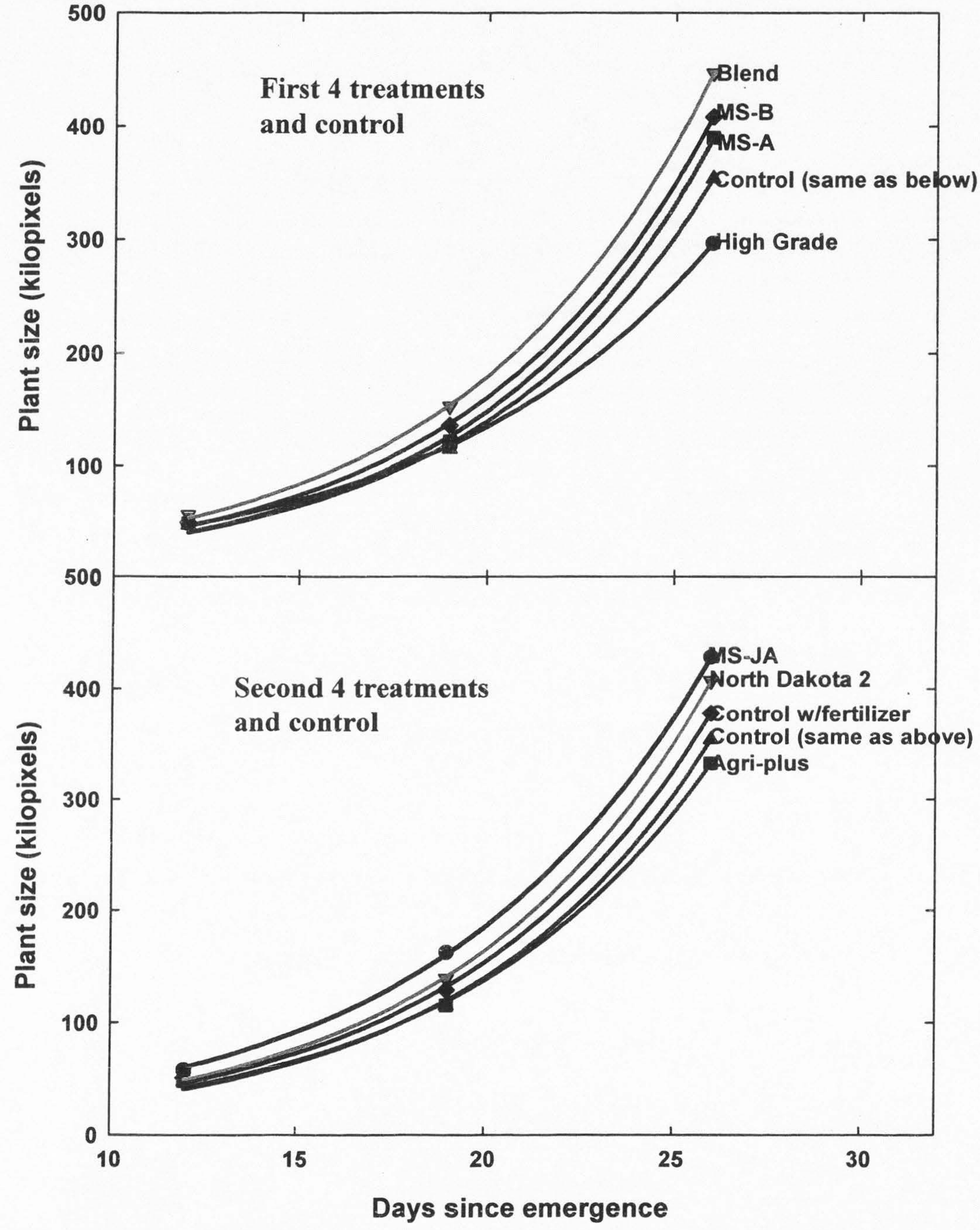


FIGURE 19. Relative growth rate curves through the first 26 days of the experiment. The control line is the same in both graphs. None of the differences are statistically significant. (Results are separated into two graphs to reduce overlap of lines).

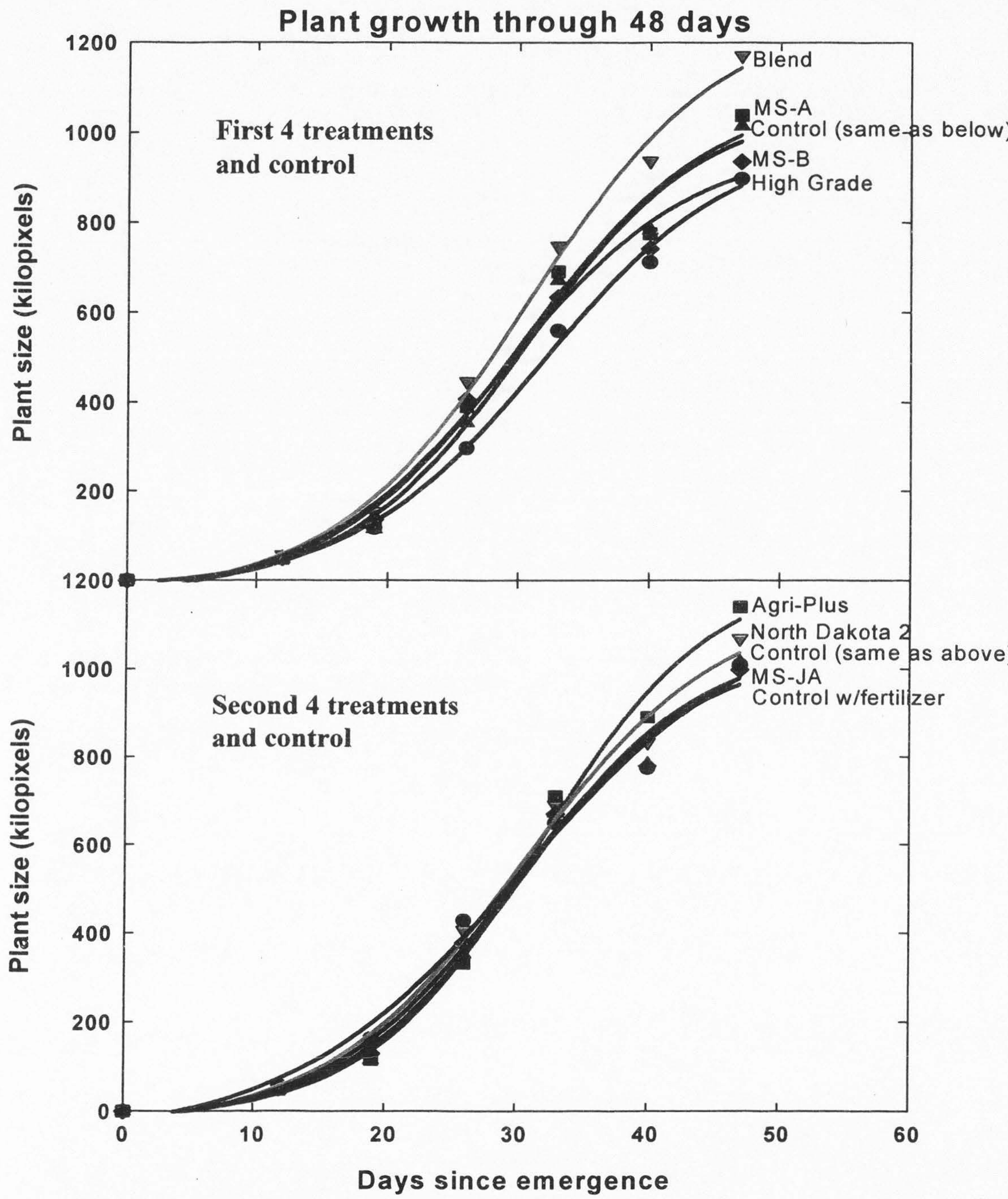


FIGURE 20. Growth rate curves for the treatments through 48 days. The control line is the same in both graphs. None of the differences are statistically significant. (Results are separated into two graphs to reduce overlap of lines).

There were no significant differences in the number of fruit per plant, fruit mass per plant, mass of fruit, or the percent fresh mass. There was also no difference in the percent of fruit that were red by number or mass. However there was a significant difference in the root growth, where the Blend did better than the control (Table 16, Figure 21).

TABLE 16. Harvest data from the experiment with the statistical analysis.

	total data for all fruit				% red fruit		
Treatment	**fruit/ plant**	**mass/ plant**	**mass/ fruit**	**% fresh mass**	**by number**	**by mass**	**root growth**
Blend	10.7	72.7	6.8	6.9	0.83	0.94	4.67a
Control	10.0	74.9	7.6	6.9	0.86	0.95	2.88b
HG 70%	11.5	73.9	6.6	6.3	0.68	0.84	4.00ab
MS-A	10.0	70.1	6.8	6.9	0.71	0.86	2.75b
MS-B	9.3	69.3	7.3	7.0	0.80	0.90	3.25b
MS-JA	10.0	73.6	8.0	7.0	0.84	0.96	3.25b
ND	9.0	71.8	8.1	6.5	0.76	0.86	3.75ab
Agri-plus	10.8	86.2	8.1	7.0	0.98	0.99	3.50ab
F-Value	0.31	0.25	0.73	0.49	0.85	1.70	2.39
Probability	0.95	0.97	0.65	0.83	0.56	0.15	0.05*
Coeff Var	28.9	28.6	19.7	8.0	25.4	9.4	23.7

* Shows statistical significance at the $\alpha = 0.05$ level.

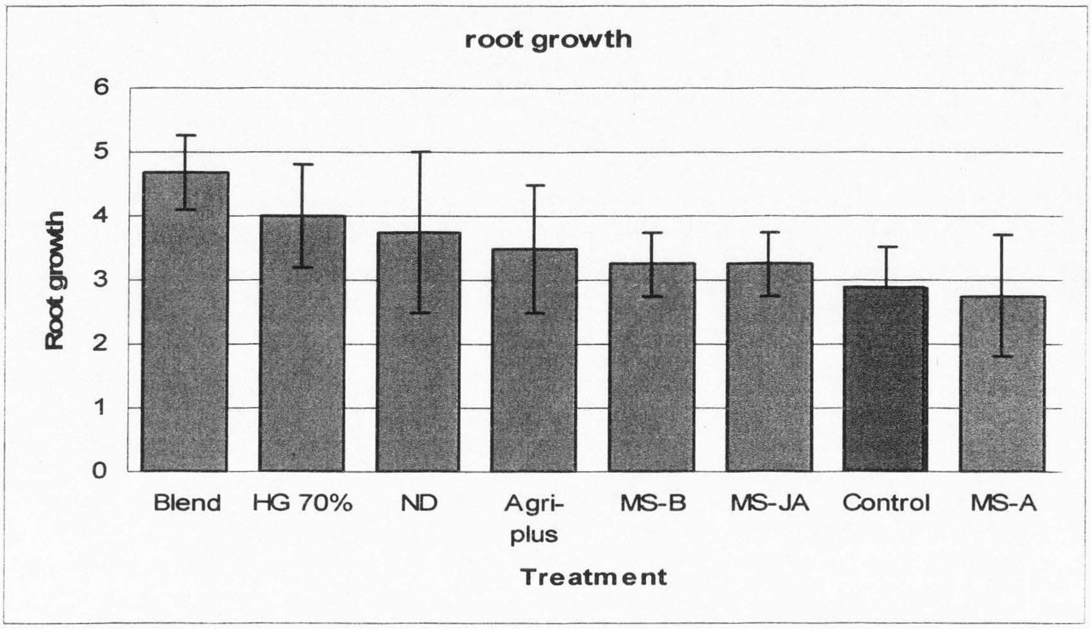


FIGURE 21. Root growth among treatments, the "Blend" treatment was significantly better than the control.

DISCUSSION

In experiment one, the treatments that produced the least red fruit tended to produce the most green fruit. Because of this there were no large differences in total fruit number. HG 70%, Luscar, MS-JA, and ND leonardite were the treatments that produced more red fruit and more total fruit mass than the control. Aldrich produced more total fruit mass than the control, but red fruit mass was the same as the control. Agri-plus produced more red fruit than the control, but in total fruit it was close to the control.

There was some difference in the average mass of each fruit, with ND leonardite and Aldrich producing the largest fruit. There were also differences in the number of fruit produced between the treatments. Number of fruit tended to increase as fruit size decreased and vise versa.

Treatments HG 70%, MS-JA, and ND leonardite were all carried over to experiment two because they had greater fruit mass than the control, but the difference was not statistically significant. Agri-plus was carried over because it tended to produce more red fruit than the control. Treatments MS-A was also were carried over from the first experiment, these carry over treatments were done to see if the results were repeatable. A fresh batch of ND leonardite (ND leonardite 2) was used in experiment two to make sure it was not an aging process that caused the effects in the first experiment. Organic acids tend to become more oxidized over time.

The only difference observed on yield in experiment two was the Agri-plus treatment produced more fruit mass, but it was not significant. These results imply that the differences in the first experiment were random chance and not treatment effects. The new treatments added to this experiment did not have beneficial affects on fruit production.

The roots were evaluated for depth in the column and penetration throughout the soil column (this was not done in the previous experiment), and there was a significant difference between the "Blend" treatment and the control. The "Blend" product may improve root proliferation, but this did not increase shoot growth or fruit production. There was no nutrient analysis done in either of these experiments.

In this soil study there were no effects seen on plant growth, similar to what Pilanali and Kaplan (2003) found. Unlike them, roots were analyzed and one treatment was found that improved root growth. Improved root growth corresponds with research mentioned in the introduction.

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CHAPTER 4

THE EFFECTS OF LIQUID HUMIC AND FULVIC ACIDS APPLIED WITH PREBAND FERTILIZER ON TOMATO GROWTH AND YIELD

ABSTRACT

There is little research on the effects of humic substances on plant growth in soil. There is also little research on what types of humic compounds are most effective. In this project, 10 commercially available liquid humic substances from Horizon Ag Products (Modesto, CA), were tested. Several of them had beneficial effects on tomato growth when they were supplied at 50 ml/liter. Treatment BA 6.6% increased fruit number, fruit dry mass and plant dry mass. Treatments QH 6.6% and Charger also increased plant dry mass. In addition fruit dry mass was increased by QH6.6%, Hydra-Hume 6% and F-6000 over control. This rate of application is extremely high and not economically feasible. In a second experiment the same 10 products were applied at 4.6 μl/liter (10,000 times lower than the first experiment). There were no significant differences in this experiment, probably because this level is to low to show effects. Other research has observed beneficial results in soil with application rates at 20 to 400 times higher than our second experiment.

INTRODUCTION

A lot of research with humic substances has used liquid materials but few have used soil as the growth media. Sanchez-Sanchez et al. (2002) used a liquid fulvic acid with EDDHA on lemon trees grown in the field. They found that the fulvic acid increased pH, Equatorial diameter, fresh mass and vitamin C of fruit that ripened in a cold chamber, when compared to EDDHA alone. Fulvic acid only increased pH and fresh mass when the fruit was allowed to ripen on the tree. The fulvic acid also increased Fe and Cu uptake in the leaves. The main point of interest is that a liquid fulvic acid had an effect on yield.

MATERIALS AND METHODS

Experiment 1

Tomato plants (cv. Red Robin) were grown in 7.6 cm (3") diameter columns made of PVC pipe cut to a length of 46 cm (18") with a PVC cap on the end. A drain hole in the middle of the cap was covered with two layers of 16-mesh screen to keep soil in the column. The columns were prepared by mixing a sandy loam soil from California with an equal volume of sand to get a 50/50 soil/sand mixture. The soil characteristics were:

Texture	Sandy Loam	
pH	7.7	
EC	1.7	
Organic Matter (%)	2.4	Walkley-Black
$CaCO_3$ (%)	8.0	
P (mg/kg)	12.4	Olsen $NaHCO_3$
K (mg/kg)	>400	Olsen $NaHCO_3$

The humic substance products tested were:

QH 6.6%

QH 12%

BA 6.6%

Hydra-Hume 6%

Charger HA

QH Oxidized 12%

Landview 7%

F-6000

VK F-6000

LMWFA HA/FA

The controls were:

No HA with fertilizer

No HA with out fertilizer

A pre-band fertilizer at 30 gallons/acre of 10-34-0 fertilizer was mixed with the humic acid products to get a 10% humic solution (1:10 v/v ratio, 3 gal HA/ac.). Each mixture was placed into three replicate columns approximately 5 cm (2 inches) below the soil surface. The columns were arranged in a randomized complete block design. The columns were started by soaking the soil with tap water then placing the seeds on top of the soil. The seeds were covered by a thin layer of vermiculite to keep them moist and improve germination. Approximately 10-15 seeds were started in each column, and thinned down to one uniform plant over the two weeks following emergence. The plants were grown in a greenhouse with supplemental light from high pressure sodium lamps. They were grown with a day/night cycle of 16/8 hours. All columns were watered with tap water mixed with 5% humic solutions (1:20 v/v ratio; 50ml/l) as needed, approximately every two to three days. The columns were leached weekly to recharge column water content.

Digital pictures were taken of the plants each week. Pixel counts were measured (using Adobe Photoshop 6.0) to calculate relative growth rates. No pictures were taken after the 33rd day because the layering of the leaves made this method less valuable.

Fruit number and fresh and dry weights were measured as fruit became ripe. The plants were harvested on the 65th day of the experiment. At the end of the experiment all ripe and unripe fruit were harvested, as were the plants. Roots were judged on a scale from one to five with five being the best, depending on how far down the column root growth had progressed, and on how well the roots infiltrated the entire column. All statistical analyses were done by an ANOVA test using SAS software (Version 9.0).

Experiment 2

The experiment set up was the same as experiment one with the addition of ceramic cups inserted into the side of the column about 5 cm from the bottom. A suction of 4 psi was pulled through the ceramic cup to draw excess water out of the column. The ceramic cups are 2.50" long with a diameter of 0.50" and an inside diameter of 0.32". The bubbling pressure of the ceramic cups is 1 Bar (Figure 22). The columns were filled with a sandy loam soil from Trenton, UT with the following soil characteristics:

Texture	Sandy Loam	
pH	7.9	
EC	0.6	
Organic Matter (%)	1.9	Walkley-Black

$CaCO_3$ (%)	5.6	
P (mg/kg)	32	Olsen $NaHCO_3$
K (mg/kg)	415	Olsen $NaHCO_3$
N (mg/kg)	6.82	$Ca(OH)_2$ extract

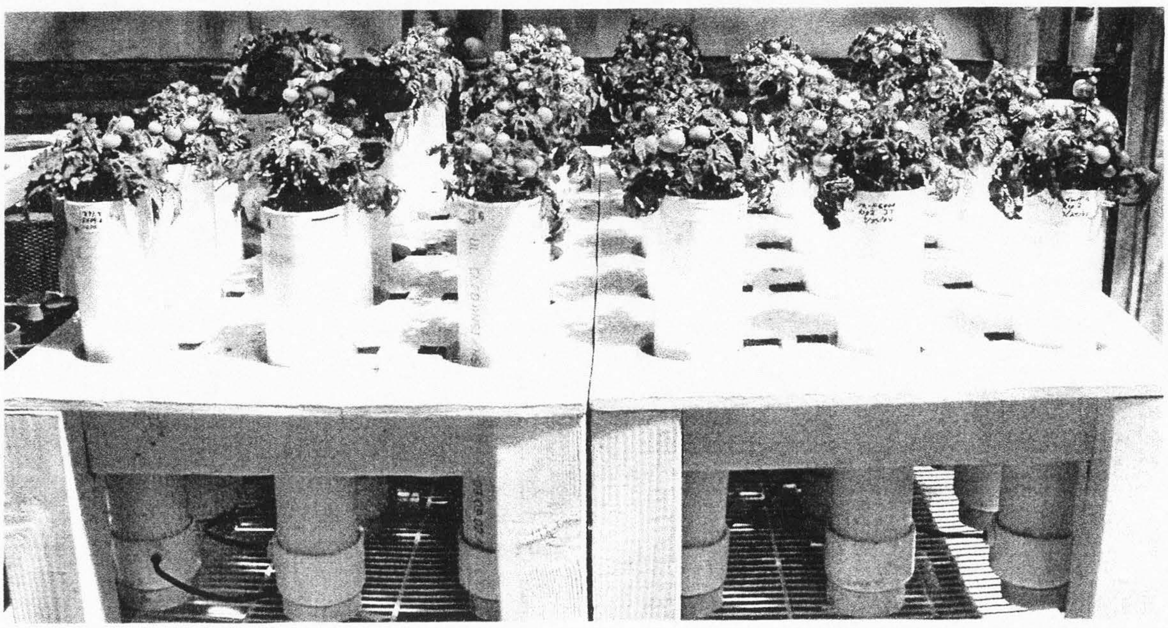

FIGURE 22. Tomato plants grown in soil columns for the liquid study experiment two. Notice black tubing at the bottom of the columns to pull suction through the ceramic cups.

The liquid humic substances products tested were:

QH 6.6%

QH 12%

BA 6.6%

Hydra-Hume 6%

Charger HA

QH Oxidized 12%

Landview 7%

F-6000

VK F-6000

LMWFA HA/FA

The controls were:

Fertilizer (no HA)

No Fertilizer (no HA)

The HA products were applied with watering througout the experiment at the rate of 4.6 μl/liter (calculated with assumption of 2 acre feet/season) mixed with tap water. In this experiment the columns were saturated from the bottom before the seeds were placed on the soil surface. Pictures were taken until the 50th day of the experiment. A majority of the plant growth happened before day 30 (Figure 23).

The plants were harvested and on the 74th day of the study. The fruit was separated into ripe (red) and unripe (green) groups. The roots were evaluated by two persons and the scores averaged for the statistical analysis.

RESULTS

Experiment 1

One replicate from the Charger treatment had stunted growth early in the experiment; as a result it did not represent the treatment so it was removed from the study. All three replicate plants for the Landview treatment died by the 19th day of the study for unknown reasons (Fig. 24). The Landview treatment was not used in any of the statistical analysis.

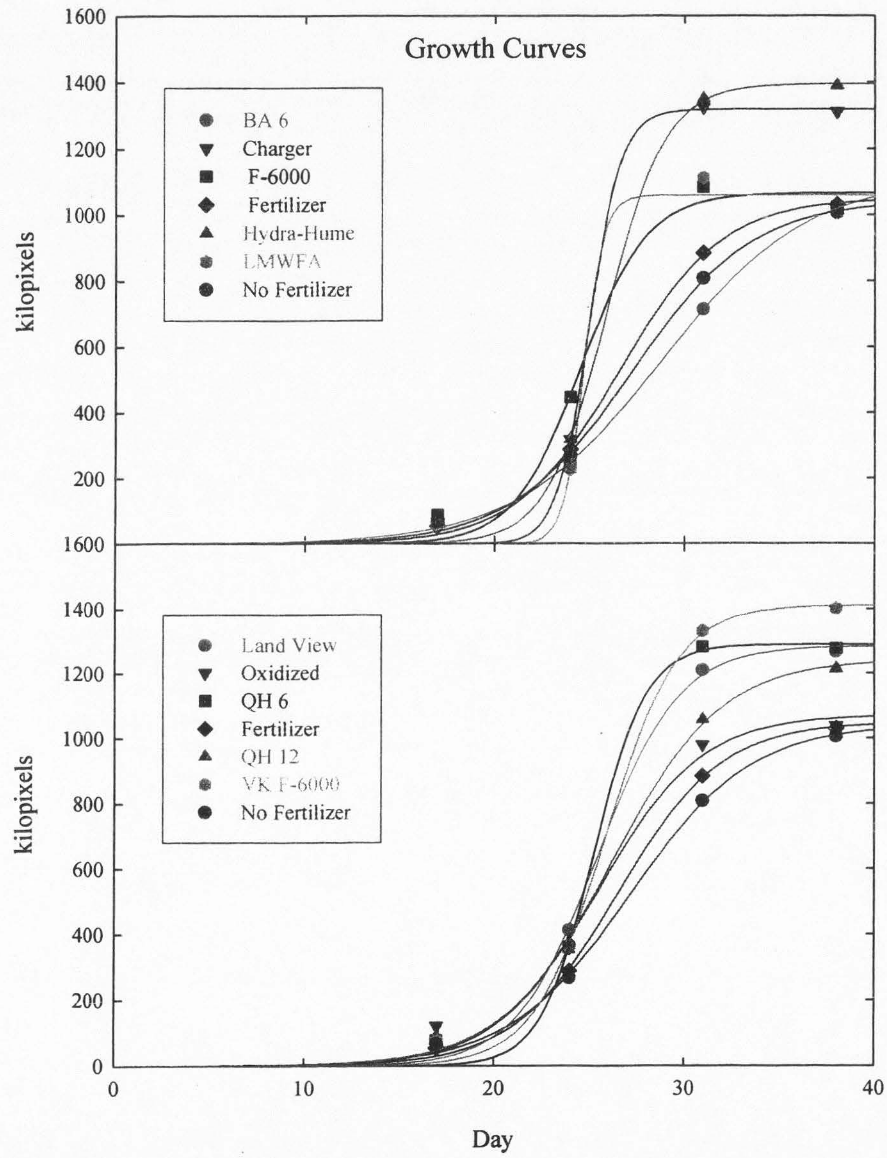

FIGURE 23. Plant growth curves, half of the treatments shown on each graph. The two controls (Fertilizer and No Fertilizer) are on both curves. Statistical differences are shown in Tables 18 to 21.

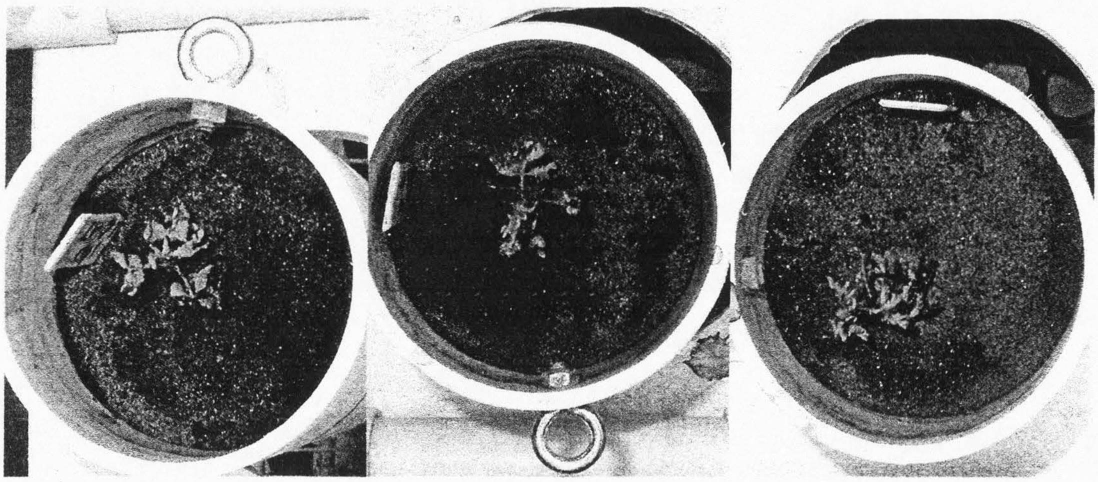

FIGURE 24. Pictures of the dead plants from the Landview treatment on the 19th day after planting.

The humic acid products were applied at very high rates in this experiment (approximately 10000 times the suggested rate, about 30 times higher than other studies). The high rate of HA application resulted in significant differences in tomato growth and yield (Table 17). Results for each product are compared to the control with fertilizer treatment. The BA and Hydra-Hume treatments produced more total fruit than the control. The BA, Hydra-Hume, QH 6%, F-6000 and Charger treatments produced more fruit mass than the control. BA, QH 6%, and Charger treatments had larger plants than the control treatment. Only the Landview treatment was statistically worse than the control in any of the measured results. There were no statistically significant differences in root growth (data not shown).

TABLE 17. Average treatment values for combined fruit number, combined fruit mass and plant dry mass, with statistical analysis.

Treatment	Combined Fruit Number		Combined Fruit Dry Mass		Plant Dry Mass	
BA	10.0	a	4.50	a	5.09	a
Hydra	9.0	ab	3.50	ab	3.09	c
QH6	8.0	abc	3.57	a	4.33	b
Charger	6.5	bcd	2.92	bcd	3.82	b
F-6000	6.0	bcd	3.05	bc	3.04	c
Control with fertilizer	5.7	bcd	1.96	d	2.61	c
QH12	5.3	cd	2.20	cd	2.87	c
Control no fertilizer	5.0	cd	2.16	cd	2.39	c
Oxidized	4.7	cd	2.44	cd	2.87	c
VK	4.3	d	2.21	cd	2.49	c
LMWFA	4.0	d	2.02	cd	2.41	c
F Value	3.5		6.9		13.68	
Pr > F	0.0071		0.0001		<.0001	
Coeff Var	29.2		19.3		12.8	

Experiment 2

The oxidized treatment was significantly larger than the Control the first week that pictures were taken (Table 18). This difference completely disappeared by the following week when all the treatments were statistically equal (Table 19).

TABLE 18. Average pixel counts on the 14th day of the experiment, with statistical analysis.

14th Day		
Oxidized	121.9	a
Hydra-hume	102.0	ab
QH 12	90.7	ab
F-6000	90.4	ab
Landview	79.4	ab
QH 6	78.5	ab
VK F-6000	76.9	ab
No Fertilizer	68.4	ab
Charger	63.8	ab
BA	60.3	ab
LMWFA	59.5	ab
Fertilizer	56.0	b
F Value	1.07	
Pr > F	0.42	
Coeff Var	40.7	
Peat Perlite	86.4	
No Suction	58.5	

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE 19. Average pixel counts on the 21st day of the experiment, with statistical analysis.

21st Day		
Hydra-hume	456	a
F-6000	448	a
Landview	414	a
QH 12	377	a
VK F-6000	364	a
QH 6	361	a
Oxidized	360	a
Charger	318	a
Fertilizer	289	a
No Fertilizer	269	a
BA	246	a
LMWFA	229	a
F Value	1.08	
Pr > F	0.42	
Coeff Var	34.8	
Peat Perlite	360	
No Suction	241	

* Treatments with different letters are significantly different at the $p = 0.05$ level.

The third week (day 28) Hydra-Hume, VK F-6000, Charger, and QH 6.6% treatments were all significantly larger than the fertilized control, the no fertilizer control and BA 6.6% treatments (Table 20). Landview was statistically better than the no fertilizer and the BA 6.6% treatments. In addition LMWFA and F-6000 treatments were significantly larger than BA 6.6%.

TABLE 20. Average pixel counts on the 28th day with statistical analysis.

28th Day		
Hydra-hume	1350	a
VK F-6000	1332	a
Charger	1324	a
QH 6	1281	a
Landview	1211	ab
LMWFA	1113	abc
F-6000	1085	abc
QH 12	1057	abcd
Oxidized	981	abcd
Fertilizer	885	bcd
No Fertilizer	809	cd
BA	715	d
F Value	3.85	
Pr > F	0.003	
Coeff Var	17.3	
Peat Perlite	1071	
No Suction	905	

* Treatments with different letters are significantly different at the p = 0.05 level.

In the fourth week VK F-6000, Hydra-Hume, Charger, and QH 6.6% treatments were all significantly larger than Oxidized, fertilized control, F-6000, BA 6.6%, no fertilizer and LMWFA treatments (Table 21). Pixel count data was collected for two more weeks, but the plants were too large so pixel counts were not valuable.

TABLE 21. Average pixel counts on the 35th day with statistical analysis.

35th Day		
VK F-6000	1402	a
Hydra-hume	1389	a
Charger	1311	a.
QH 6	1277	ab
Landview	1267	ab
QH 12	1214	ab
Oxidized	1038	b
Fertilizer	1031	b
F-6000	1024	b
BA	1018	b
No Fertilizer	1007	b
LMWFA	1006	b
F Value	3.91	
Pr > F	0.003	
Coeff Var	11.7	
Peat Perlite	1652	
No Suction	1302	

* Treatments with different letters are significantly different at the p = 0.05 level.

The plant dry mass data does not completely correlate with the pixel count data. Treatments QH 6.6% and Hydra-Hume had statistically more mass than F-6000 (Table 22).

TABLE 22. Plant dry mass treatment means and statistics.

Plant dry mass		
QH 6	7.81	a
Hydra-hume	7.74	a
VK F-6000	7.58	ab
Landview	7.44	ab
Fertilizer	7.34	ab
Charger	7.23	ab
QH 12	7.12	ab
No Fertilizer	5.87	ab
LMWFA	5.76	ab
BA	5.69	ab
Oxidized	5.45	ab
F-6000	5.14	b
F Value	1.89	
Pr > F	0.096	
Coeff Var	18.6	
Peat Perlite	17.9	
No Suction	7.5	

* Treatments with different letters are significantly different at the p = 0.05 level.

Ripe fruit did not show any treatments effects for fruit number or fresh mass (Table 23). In addition there was no treatment effect on mass per fruit (Table 24). Dry mass did have some differences with the Charger treatment being significantly larger than the fertilized control, LMWFA, Oxidized and Landview treatments (Table 24).

TABLE 23. Red fruit treatment means and statistically analysis for fruit number and fresh mass.

Red fruit number		
Hydra-hume	11.7	a
F-6000	11.7	a
Charger	11.3	a
VK F-6000	11.0	a
QH 6	10.0	a
LMWFA	9.5	a
BA	9.3	a
QH 12	9.3	a
Oxidized	9.3	a
No Fertilizer	9.0	a
Fertilizer	9.0	a
Landview	8.7	a
F Value	0.83	
Pr > F	0.62	
Coeff Var	21.2	
Peat Perlite	16.0	
No Suction	7.7	

Red fruit fresh mass		
VK F-6000	80.1	a
Charger	74.5	a
Hydra-hume	74.0	a
QH 6	72.5	a
QH 12	71.4	a
F-6000	71.0	a
BA	69.9	a
Oxidized	67.7	a
Fertilizer	66.9	a
No Fertilizer	66.6	a
Landview	64.1	a
LMWFA	63.2	a
F Value	0.47	
Pr > F	0.91	
Coeff Var	16.8	
Peat Perlite	198.0	
No Suction	56.8	

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE 24. Red fruit treatment means and statistically analysis for mass per fruit and dry mass.

Red mass/fruit			**Red fruit dry mass**		
QH 12	7.69	a	Charger	5.76	a
BA	7.65	a	VK F-6000	5.72	abc
Oxidized	7.53	a	Hydra-hume	5.56	abc
No Fertilizer	7.45	a	QH 12	5.19	abc
Fertilizer	7.44	a	QH 6	4.99	abc
Landview	7.41	a	BA	4.59	abc
QH 6	7.40	a	F-6000	4.53	abc
VK F-6000	7.31	a	No Fertilizer	4.40	abc
LMWFA	6.76	a	Fertilizer	4.27	bc
Charger	6.59	a	LMWFA	4.26	bc
Hydra-hume	6.42	a	Oxidized	4.19	c
F-6000	6.08	a	Landview	4.19	c
F Value	1.07		**F Value**	2.02	
Pr > F	0.42		**Pr > F**	0.075	
Coeff Var	12.4		**Coeff Var**	15.3	
Peat Perlite	12.50		Peat Perlite	11.10	
No Suction	8.00		No Suction	3.58	

* Treatments with different letters are significantly different at the p = 0.05 level.

Combined ripe and unripe fruit data had no differences in fruit number, fruit fresh mass, fruit dry mass, or mass per fruit (Tables 25 & 26).

TABLE 25. Total fruit treatment means and statistically analysis for fruit number and fresh mass.

Total fruit number		
Fertilizer	20.7	a
Hydra-hume	18.3	a
Landview	17.7	a
VK F-6000	17.0	a
Charger	16.7	a
QH 12	16.7	a
F-6000	16.3	a
Oxidized	15.0	a
QH 6	15.0	a
LMWFA	14.5	a
BA	14.3	a
No Fertilizer	11.7	a
F Value	0.5	
Pr > F	0.88	
Coeff Var	34.2	
Peat Perlite	53.0	
No Suction	12.0	

Total fruit fresh mass		
Fertilizer	107.9	a
VK F-6000	98.0	a
Charger	93.4	a
Landview	93.2	a
QH 6	91.0	a
Hydra-hume	90.9	a
QH 12	90.7	a
F-6000	89.8	a
BA	89.6	a
Oxidized	88.0	a
LMWFA	82.5	a
No Fertilizer	75.5	a
F Value	0.48	
Pr > F	0.90	
Coeff Var	21.1	
Peat Perlite	457.0	
No Suction	72.7	

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE 26. Total fruit treatment means and statistically analysis for mass per fruit and dry mass.

Total mass/fruit			**Total fruit dry mass**		
No Fertilizer	6.49	a	Charger	7.27	a
Oxidized	6.47	a	VK F-6000	7.21	a
BA	6.30	a	Hydra-hume	7.12	a
QH 6	6.28	a	Fertilizer	7.07	a
VK F-6000	5.89	a	QH 12	6.68	a
Charger	5.73	a	Landview	6.42	a
LMWFA	5.67	a	QH 6	6.41	a
QH 12	5.67	a	BA	6.01	a
Landview	5.64	a	F-6000	5.85	a
F-6000	5.50	a	LMWFA	5.63	a
Hydra-hume	5.43	a	Oxidized	5.58	a
Fertilizer	5.30	a	No Fertilizer	5.07	a
F Value	0.48		**F Value**	0.95	
Pr > F	0.90		**Pr > F**	0.51	
Coeff Var	17.8		**Coeff Var**	20.1	
Peat Perlite	8.62		Peat Perlite	25.40	
No Suction	5.80		No Suction	4.41	

* Treatments with different letters are significantly different at the p = 0.05 level.

The root analysis found that F-6000 and Oxidized treatments were significantly better than QH 6.6% and Charger (Table 27). LMWFA and Hydra-Hume were also better than the Charger treatment.

TABLE 27. Root growth treatment means and statistics.

Root growth		
F-6000	4.50	a
Oxidized	4.42	a
LMWFA	4.25	ab
Hydra-hume	4.25	ab
Fertilizer	4.08	abc
BA	4.08	abc
QH 12	3.92	abc
VK F-6000	3.83	abc
Landview	3.83	abc
No Fertilizer	3.75	abc
QH 6	3.50	bc
Charger	3.33	c
F Value	1.82	
Pr > F	0.110	
Coeff Var	11.3	
Peat Perlite	4.42	
No Suction	3.08	

* Treatments with different letters are significantly different at the p = 0.05 level.

DISCUSSION

At extremely high rates of application in experiment one, which are not economically feasible, none of the HA products tested had noticeable detrimental affects, with the exception of Landview. A few of the products showed improved plant growth and yield over the control, particularly the BA 6% and Charger HA. Hydra-Hume 6% treatments also had increased yield over the control.

In experiment two, the economically feasible rate was used and no beneficial statistically significant results were observed when compared to the fertilized control. It appears that the economical rate is not adequate to create beneficial results.

Fagbenro and Agboola (1993) growing plants in soil observed beneficial effects beginning at a rate about 20 times what was used in this experiment. They continued to see benefits up to a rate roughly 330 times higher than experiment two. Future work done on these liquid products should use a rate lower than the first experiment but higher than the second.

The addition of the ceramic cups to the bottom of the columns did not increase root growth to the expected amount. This lack of ideal root growth did not appear to have a detrimental effect on shoot growth or fruit production. The two treatments with the smallest roots, Charger and QH 6.6 %, were some of the largest plans according to pixel count data and plant dry mass. Charger also produced the most ripe fruit dry mass. At the same time most of the treatments with the best root growth were in the bottom half of the pixel count data and the bottom of plant dry mass. The smallest plants in this experiment appear to have grown extensive root systems in an attempt to get adequate shoot growth. The only treatment that had above average root and shoot growth was Hydra-Hume.

Columns with no suction and peat-perlite columns were grown alongside experiment two. The peat-perlite columns had much better growth and production (Tables18 – 27). For example, plant dry mass was 17.9 g (>2x better than next treatment), ripe fruit fresh mass 198.0 g (2.5x better than top treatment) and total fruit fresh mass 456.7 g (4x better than top treatment). This suggests that the experimental procedure did not provide adequate growth conditions. It is likely that the plants were flood stressed even with the suction pulled through the ceramic cups. The suction brings the columns to field capacity in 24-48 hours. Soil typically has about 10% available water based on volume, with these columns having a total of 2 liters volume the available water is approximately 200 ml. A large plant could us this amount of water in a 24 hour period.

The plants cycled between water logged and drought conditions throughout this experiment. The plants were overly stressed. To solve this problem the columns should be lengthened to approximately double their current length. The extra length would double the available water and it would draw excess water down to the lower portions of the column and away from the roots; improving conditions for plant growth.

A change of plants may also be warranted. The current system was tested using maize plants which grew equally well in both soil and peat perlite columns. However, this has not been the case with tomatoes.

REFFERNCES

Fagbenro, J.A., and A.A. Agboola. 1993. Effect of different levels of humic acid on the growth and nutrient uptake of teak seedlings. J. Plant Nutrition 16:1465-1483.

Sanchez-Sanchez, A., J. Sanchez-Andreu, M. Juarez, J. Jorda, and D. Bermudez. 2002. Humic substances and amino acids improve effectiveness of chelate FeEDDHA in lemon trees. J. Plant Nutrition 25:2433-2442.

CHAPTER 5

THE EFFECT OF "ASSAULT" ORGANIC AMENDMENT (AOM) ON WHEAT AND ALFALFA GROWN WITH SALINE WATER

ABSTRACT

Organic compounds have high cation exchange capacities, meaning they may be able to reduce salt effects that are introduced to a field through irrigation water. In the current study both a liquid and dry granular form of Assault Organic Amendment (AOM) from Horizon Ag Products were tested to see if they can improve plant growth when they are irrigated with high salinity water. The liquid product was applied at 30 and 100 μl/liter. The dry granular product at 100 and 200 lbs/acre. There was a combination treatment that used 100 lbs/acre dry and 100 μl/liter liquid. None of the treatments tested helped reduce salt stress and improve plant growth.

INTRODUCTION

There are 30,000 coal bed methane wells in Wyoming and Montana that produce water with varying levels of salts. The dominant salt is sodium bicarbonate. Although the water is useful resource for an otherwise arid region, the salt load must be managed to avoid excessive concentrations of salts in the soils, aquifers and streams of the region. Assault organic amendment has been proposed as a tool to minimize degradation of soils and yield losses from excessive sodium bicarbonate salts. This study was designed to simulate the effects of irrigating wheat and alfalfa with saline water to determine the effects Assault organic amendment has on plant growth. Assault could have any of three types of beneficial effects on plant growth under these conditions: 1) bind excess salt ions, effectively removing them from the soil solution, 2) help maintain soil structure against the degrading effects of sodium, 3) chelate nutrients keeping them in the soil solution and making them available for the plants.

MATERIALS AND METHODS

Plants were irrigated with water at three salinity levels. The salinity treatments, formulated by Dr. Jim Bauder at Montana State University, were:

1) tap water (Electrical Conductivity = 0.4 dS/m, Sodium Adsorption Ratio = 3.5)

2) moderate salinity water (EC = 2.9, SAR = 13.1). Prepared by mixing;

100 L of deionized water

65 g sodium bicarbonate

10 g potassium sulfate

80 g sodium chloride

15 g magnesium sulfate

60 g calcium carbonate

30 g calcium chloride (anhydrous)

3) high salinity water (EC = 6.4, SAR = 24). Prepared by mixing;

100 L of deionized water

200 g sodium bicarbonate

25 g potassium sulfate

160 g sodium chloride

45 g magnesium sulfate

75 g calcium carbonate

91 g calcium chloride (anhydrous)

The ion concentrations for the different salinity levels are shown in Table 28. In this table and throughout the rest of the report, the tap water treatment will be referred to as low salt.

TABLE 28. Concentration of salt ions in the three salinity treatments measured in mmol/liter.

Ion	Low	Moderate	High
		mmol/l	
K	<0.03	1.14	2.88
Ca	1.08	8.70	15.7
Mg	0.58	0.61	1.84
Na	0.06	21.4	51.4
SO_4	0.15	1.18	3.28
CO_3	<0.01	13.7	31.3
Cl	<0.03	19.2	44.0

TABLE 29. Comparison of the salt water treatments being used in this study and a water sample from a well in Montana. All measurements are in mg/l. Montana well water was best represented by the high salt treatment.

Water source	Ca	K	Mg	Na	S	Si
Tap Water	43.2	<	13.9	1.36	2.55	1.78
Moderate water	5.89	6.77	6.18	580	43.9	15.9
High Salt water	2.34	95.0	38.6	1072	105	1.36
Montana Salt water	19.7	114	37.5	1145	110	0.14
Reporting Limits	0.20	1.00	0.20	0.20	0.20	0.05

In order to extrapolate the information gathered in this study to the field, water was collected from a coal bed methane well in Montana and analyzed for salt content.

This analysis was compared to the salt contents found in the treatments of this study (Table 29).

Experiment one: wheat (cv. Rick) was grown in sandy loam soil. Each salinity level had four treatments; 3 levels of Assault organic amendment and a control. There were no replicates.

1) Assault liquid at 10 ppm in irrigation water

2) Assault liquid at 100 ppm in irrigation water

3) Assault dry granular at 200 lbs. per acre

4) Control (no Assault)

Experiment two; wheat (cv. Rick) and alfalfa (cv. DKA 42-15) were grown. The number of treatments for the salinity levels was increased from four to six, and the 10 ppm treatment was increased to 30 ppm. All treatments were replicated twice.

1) Assault liquid at 30 ppm in irrigation water

2) Assault liquid at 100 ppm in irrigation water

3) Assault dry granular at 100 lbs. per acre

4) Assault dry granular at 200 lbs. per acre

5) Assault dry granular at 100 lbs. per acre plus 100 ppm liquid in irrigation water

6) Control (no Assault)

Plants were grown in 46 cm (18") long columns made of 7.6 cm (3") diameter PVC pipe with PVC caps on the end. A drain hole in the middle of the cap was covered with 16-mesh screen to keep soil in the columns. The columns were prepared by mixing a sandy loam soil (from stock at hand) with an equal amount of sand to get a 50/50 soil/sand mixture by volume. The soil characteristics were (nutrient values are available nutrients):

Texture-	Sandy Loam	
pH	7.8	
EC	2.9	
OM (%)	2.3	Walkley-Black
SAR	0.85	
$CaCO_3$ (%)	0.8	
P (mg/kg)	1239	Olsen $NaHCO_3$
K (mg/kg)	1680	Olsen $NaHCO_3$
Ca (mg/kg)	10045	saturation paste
Zn (mg/kg)	37	DTPA extraction
Cu (mg/kg)	9.6	DTPA extraction
Mn (mg/kg)	301	DTPA extraction

This sand/soil mixture was then mixed with ammonium sulfate fertilizer at a rate of 1.34 g per 50.3 cm^2 column surface area (500 lbs. per acre). A nitrogen only fertilizer was used because the soil had high native levels of phosphorus and potassium. Normal fertilization levels in the field would be 100 lbs. per acre. It was necessary to fertilize at five times this amount, because the columns restrict the volume of soil the roots can utilize compared to the field (Figure 25). Preliminary studies in our lab have shown that fertilizing at about five times standard rate give the best results.

Half of the fertilizer was mixed with the whole volume of soil and this was used to fill the columns half way. Then the other half of the fertilizer was mixed in with the remaining soil and the columns were filled the rest of the way. This got the majority of fertilizer into the top of the columns to imitate field conditions where tilling would work the fertilizer into the top 15-23 cm (6-9 inches).

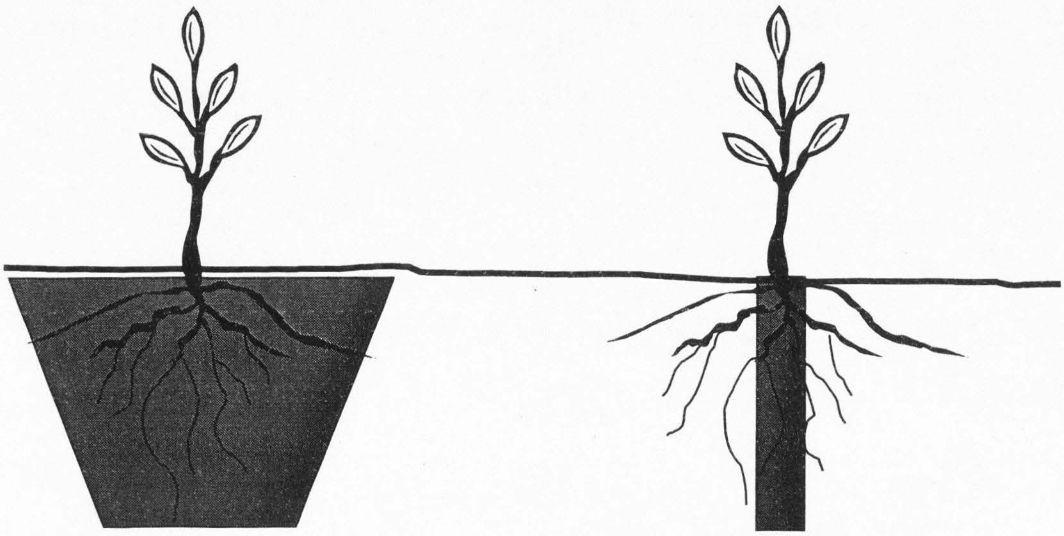

FIGURE 25. Conceptual comparison of the volume of soil utilized by plant roots in the field and the smaller volume of soil available when roots are enclosed in PVC columns. The columns required more fertilizer per unit column surface area than the field.

The dry granular Assault Organic Amendment (AOM) was mixed into the top 5 cm (2 inches) of the appropriate columns by scooping the top 5 cm (2") of soil out of the columns and mixing in the AOM at appropriate levels; 0.056 g/column or 0.112 g/column (100 or 200 lbs. per acre).

All columns were watered with tap water until plant emergence; at which time the liquid treatments were started. Plant height and chlorophyll content were measured weekly. The columns were leached weekly and the leachates were analyzed for EC and pH. The experiment ran for 40 days. Fresh and dry mass were measured at the end of the experiment. Results are the mean of three plants per column for the wheat, and two to four plants per column for the alfalfa.

RESULTS

Plant height differences resulting from variations in water quality were visually apparent (Figure 26); there was no visual difference in height for the Assault treatments. Height was not measured on the alfalfa plants.

The wheat had no significant trend in total dry mass for the liquid treatments at any salinity level (Figure 27a). The figure shows slight decreases in plant mass under low and moderate salt stress with increasing assault application. At high salt stress there was a small increase in dry mass with increasing treatment levels. There were no significant treatment effects for the dry granular products (Figure 27a). Small increases in plant mass were seen in the low and high salt treatments, a small decrease was seen in moderate salt treatments with increasing assault product. It has been suggested that second order regression lines may fit organic amendments better than first order, but this was not the case in the current study, with curves going both directions (Figure 27b).

Even though there was no significant difference in the total dry mass of the treatments it is possible that the assault products would have an effect on a particular plant part such as the leaves or heads. So the mass of each treatment was broken down into its individual above ground parts (Figure 28). There was no treatment effect seen on the leaves, stem or heads.

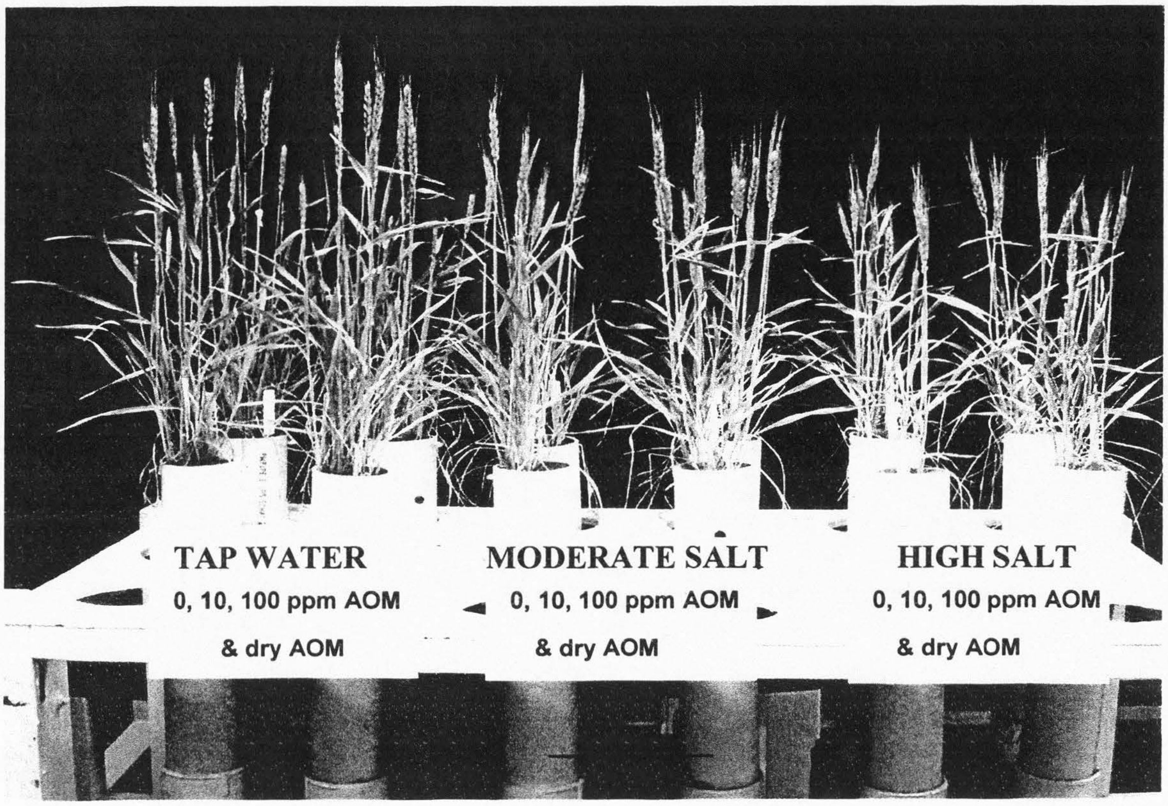

FIGURE 26. Wheat plants grown in soil columns grouped according to salt stress. Within each group the front plants starting on the left are; control, 10 ppm. The back plants from the left are 100 ppm and dry granular. AOM stands for Assault Organic Amendment.

The last step in the wheat analysis was to compare the data for the mixed liquid and dry granular treatment (shown at the right hand side of Figure 28). This treatment did not show a beneficial effect on any part of the plant dry mass.

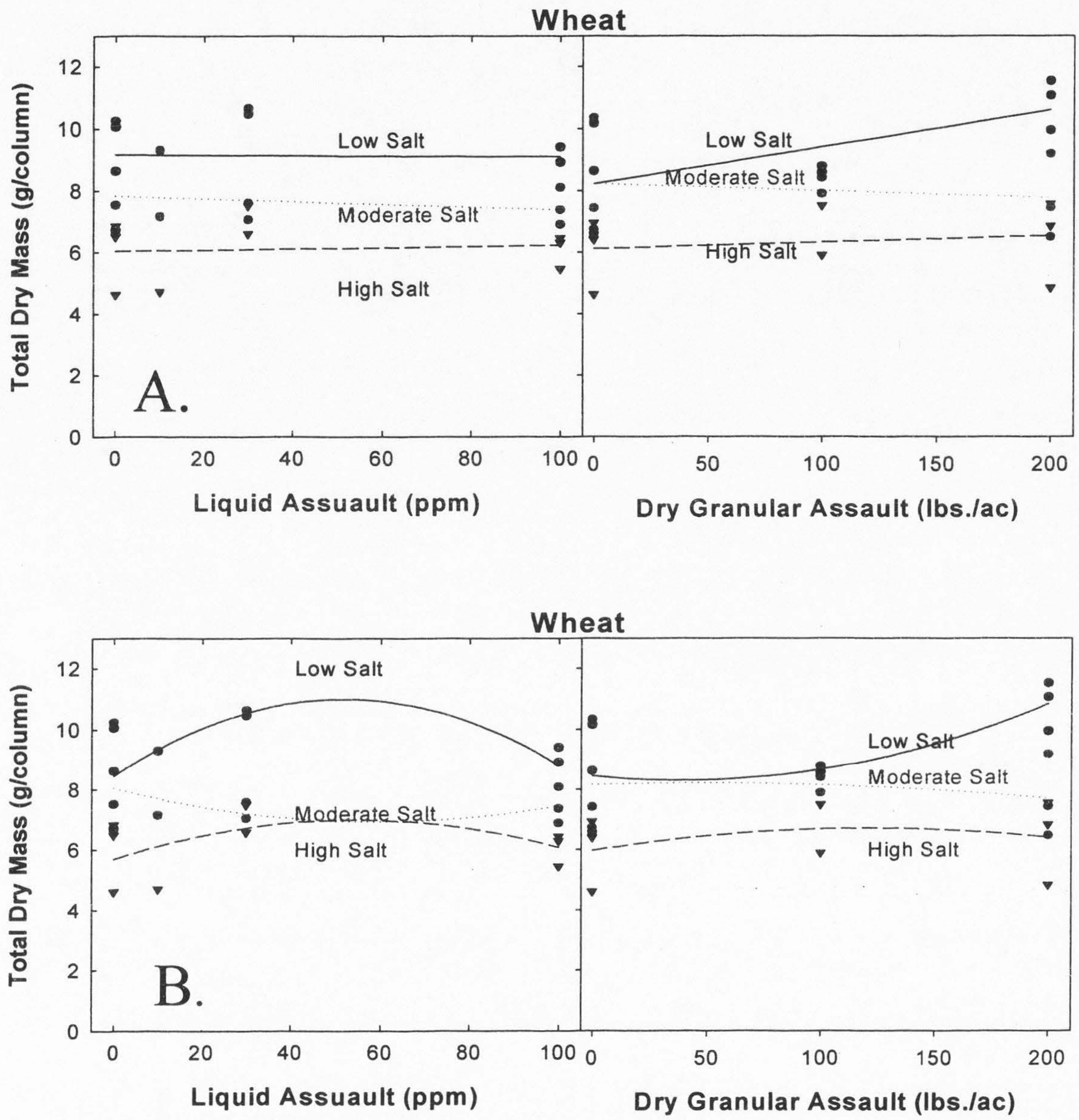

FIGURE 27. A) Wheat dry mass showing points for each column, with regression lines for the treatments. B) The same as Figure A with second order regressions lines. These graphs show data collected in both experiments.

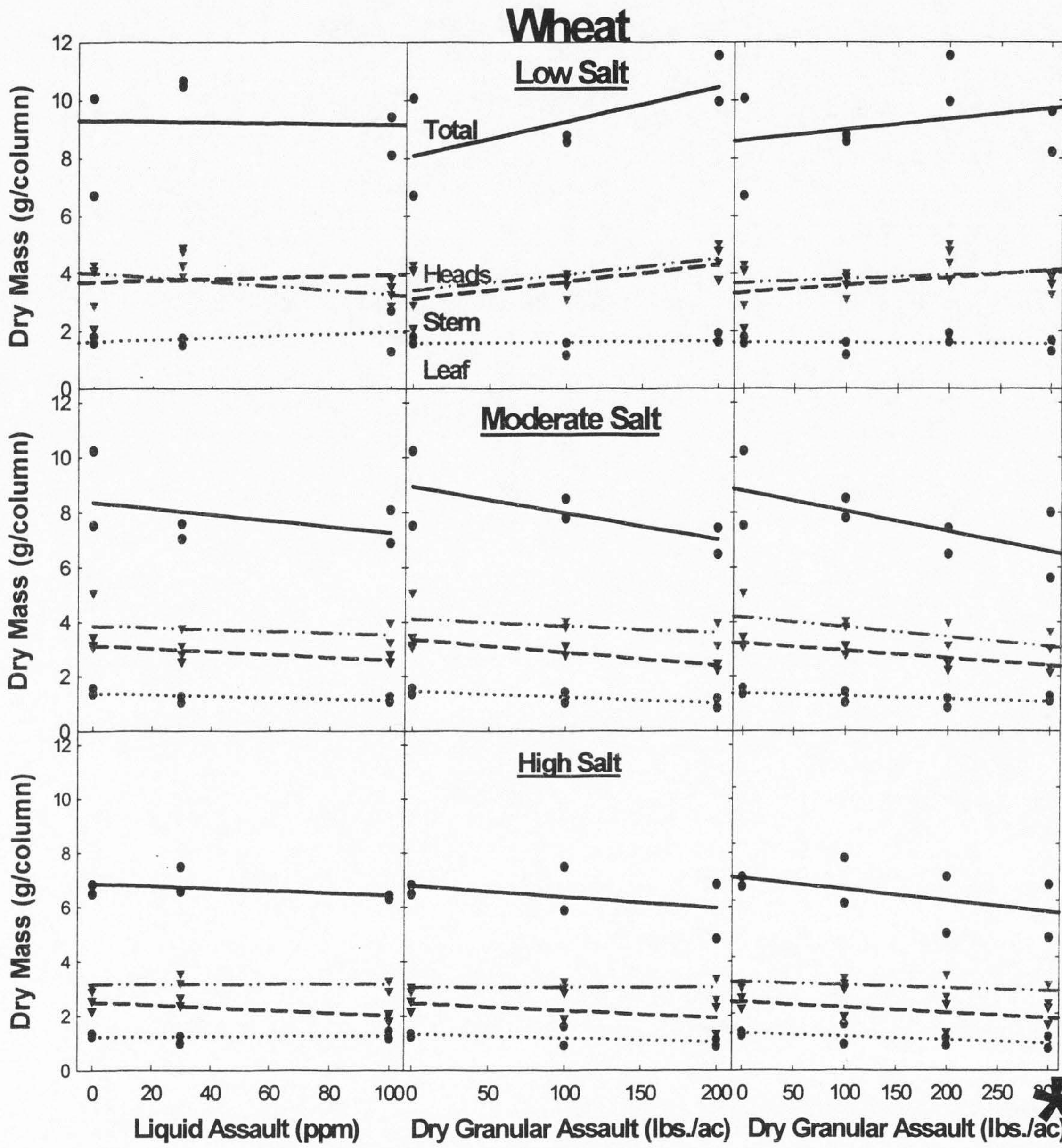

FIGURE 28. Component parts of the wheat shoot for the dry mass in the second salt experiment. The component parts include the leaf, stem and head. There was no significant difference. The third column adds the Dry Granular 100 lb/ac. plus 100 ppm liquid treatment represented by Dry Granular 300 lbs/ac (indicated by the star in the graph). The third column does not have true regression lines, but it includes the mixed treatment for comparison.

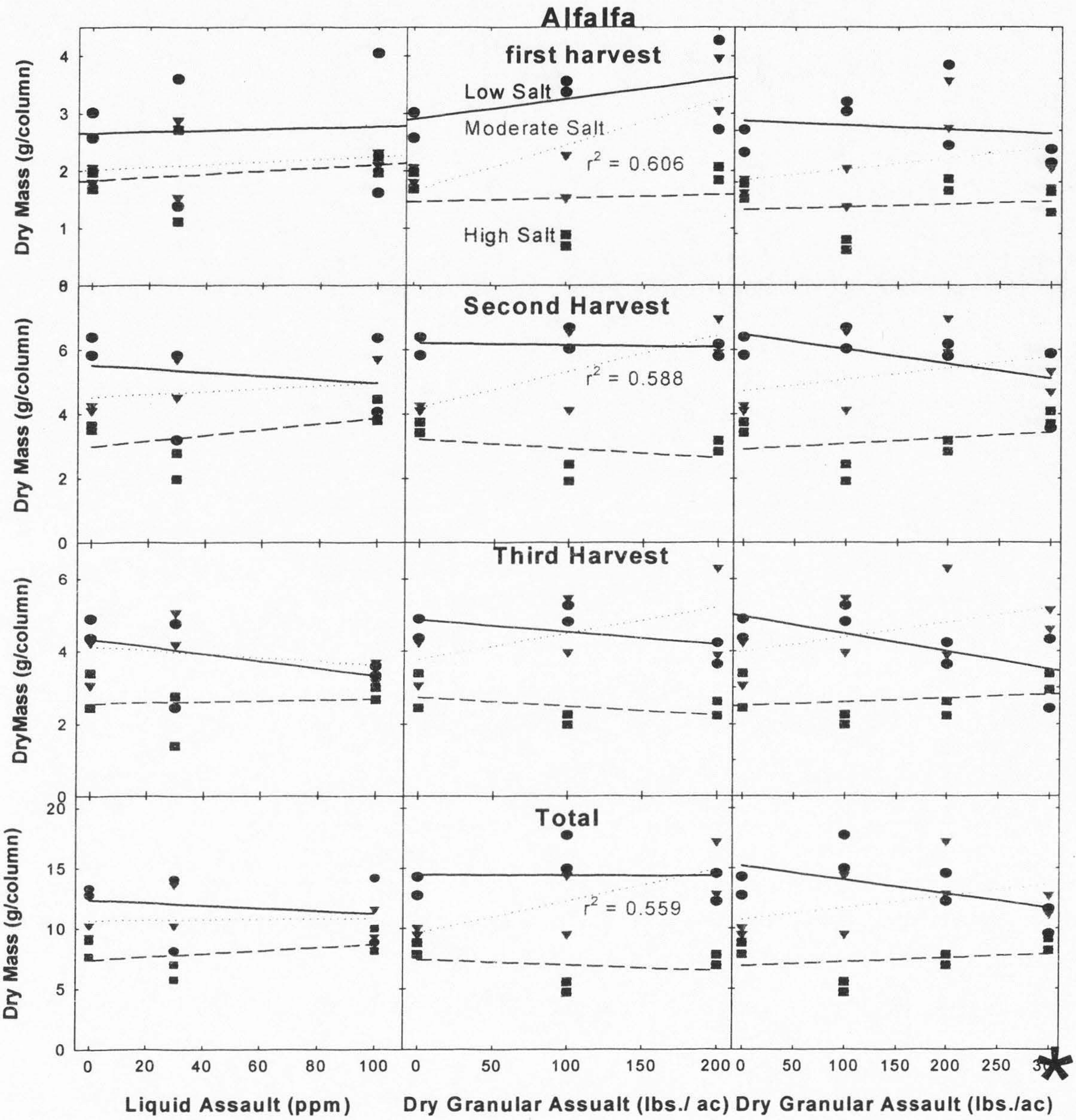

FIGURE 29. Total dry mass from first, second and third alfalfa harvests starting with first harvest on the first row (significant r^2 values shown). The bottom graphs show the harvests added together. The added treatment of 100-lbs./ac dry granular assault and 100 ppm liquid assault given the name 300 lbs./ac. (indicated by the star in the graph) The third columns does not have true regression lines.

The alfalfa data (Figure 29) does not indicate a consistent beneficial effect. The only significant treatment effect was for the dry granular treatment under a moderate salt stress. This was significant in the first and second harvest and for total mass harvested. The right hand column shows the data for the mixed liquid and dry granular treatment.

There was no significant difference among the treatments in chlorophyll content, leachate pH, or EC (data not shown).

Humic substances may improve soil structure under sodic conditions, to test this, the infiltration rates of the columns were checked at the end of the experiment (Figure 30). The treatments with 100 ppm liquid product appear to increase infiltration.

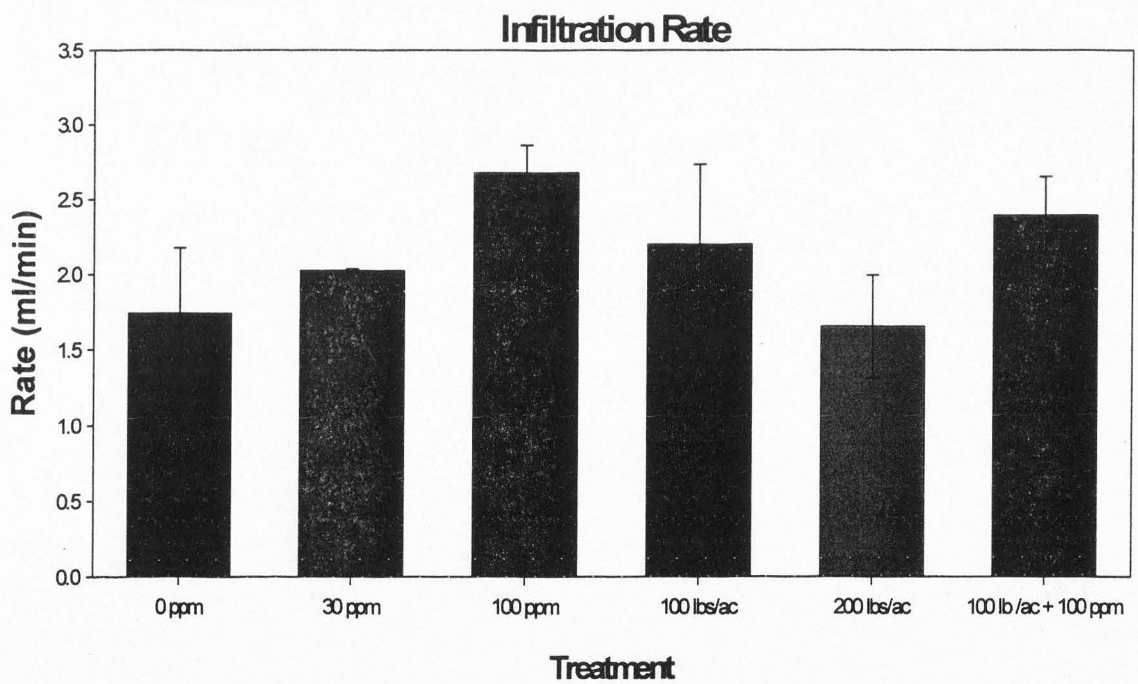

FIGURE 30. Average infiltration rates for the soil columns from the high salt stress treatments.

DISCUSSION

One of the ways the Assault organic amendment might help salt stressed plants is by binding excess salt ions and removing them from solution. To examine this claim, mass balance of salt ions in solution versus the charged sites on Assault liquid products was examined. If it is assumed that Assault liquid products have a Cation Exchange Capacity (CEC) of 500 cmol/kg (0.005 mmol/mg). At the rate of 100 ppm (100 mg/liter) Assault used in this study would give a CEC of 0.5 mmol/liter. The cations added to the moderate salt solution are 31.9 mmol/liter (Table 28), with a charge of 41.2 mmol/liter. Even the low salt water from the tap has 3.4 mmol of charge per liter. The cation charge in solution is significantly greater than the CEC of Assault products (Assault could only bind 1.2 % of cations in the moderate solution). It is doubtful that even the high CEC of Assault Organic Amendment would have an effect on the ion concentration in the soil solution.

Assault may have a beneficial effect on soil structure, and this effect may be greater in sodic environments. However, a sandy loam soil mixed with 50% sand was used in this research so the effect on soil structure would not be expected in this system. Additional studies to quantify possible beneficial effects on soil aggregation would be helpful.

Assault may also have a significant effect on improving nutrient availability. The high salt in this study could have reduced nutrient uptake, but this was generally a fertile soil with no obvious nutrient deficiencies. Further tests are needed to examine the effects of Assault on nutrient availability in soil solution. The effects on iron availability are especially important in these high pH soils.

CHAPTER 6

CONCLUSIONS

A soil system that simulates conditions of a field soil was developed to study the effects of humic substances on plant growth. A system like this is needed to answer the questions of how and why these substances help plant growth in the field. Humic substances appear to have different effects in soils than in hydroponics or sand systems.

The research reported herein, like most other research on humic substances, has not shown consistent effects on plant growth and yield. Nutrient analysis of plant tissues was not done when there was no significant effect on plant growth. Several published papers did not find beneficial effects on plant growth but they still claimed increased nutrient uptake. Many published papers reach questionable conclusions based on their data.

This research found increased iron uptake in a sand system and in hydroponics (Chapter 1 and Appendix A). This is in agreement with many other researchers. The rate of humic acid used to get beneficial effects in the sand system in Chapter 1 is far beyond what is economically feasible. This agrees with some other researchers, who suggest that these products are only economical when applied as foliar sprays (see Appendix C). In Chapter 4, beneficial effects were seen using extremely high rates of liquid humic and fulvic acids. Again this is a non-economical rate. The experiment was done at a lower rate and no effect was found. The rate of application is important in identifying treatment effects, but there is no agreed on ideal rate (Chapter 1). The lack of results reported in this paper may be a result of using low application rates.

Humic substances will probably not help bind excess salt from a saline solution (Chapter 5). There are not enough active sites to remove significant amounts of salt from solution, but they could increase soil aggregation and stability under sodic conditions. Humic substances are unlikely to have much of a beneficial effect in a system with high latent organic matter content, such as the study in Appendix D.

Humic acids can improve and maintain soil structure including flocculating clay particles. They also have many active sites that can bind plant nutrients.

Humic acids are probably not in solution in the soil environment so the plant roots would need to grow close to them to extract nutrients. However, fulvic acids are highly soluble in soil so they must bind to other organic matter, or a soil surface, to prevent being leached out of the root zone. Irrigation must thus be carefully managed before fulvic acids will improve plant growth and nutrition in the field. The best results when applying humic substances to the field would be obtained when soluble (FA) and non-soluble (HA) portions are added together.

Humic and fulvic acids have a limited ability to make unavailable mineral nutrients available to plants, however, they maybe capable of "keeping nutrients available" (generally a phrase similar to this is used in the literature). This is one of the reasons why many studies using hydroponics observe improved results, but the results are small or insignificant in soil, unless the substance has been nutrient enriched first. Humic substances may be more beneficial when soil microbes are more active. Humic substances should be able to bind substances released by microbes including many plant nutrients. Humic substances also have some reducing power when microbes are near, because some microbes can use soil organic matter as an electron acceptor in metabolism. However, this benefit may be limited to anoxic conditions, were few crops are grown.

Humic substances, especially fulvic acid, can be taken up by plants. This is where their so called hormone like responses is observed. Unfortunately none of the literature reviewed for this thesis has data to substantiate this claim.

RECOMMENDATIONS

Additional work on the chelating abilities of humic substances in hydroponic solutions and sand systems would be beneficial. There is a significant market for iron chelators and humic substances could be a cheaper alternative than the commercial chelators available today.

The soil system developed through this research could provide vital information on how these substances act in soils. The system has continued to be improved since the completion of these studies. If the question of how humic substances effects plant growth is going to be answered then a carefully designed system is necessary. Higher rates of humic substances should be used in future work to identify their effects, even if these rates are not economical. Identifying the best products for use on crops should be the ultimate objective of humic substance research because all sources and products are not the same.

Foliar studies should be conducted to substantiate the claims made by others and to determine if humic substances might be beneficial and economical when applied directly to leaves.

APPENDICES

APPENDIX A. EFFECTS OF A HUMIC ACID AND FULVIC ACID ON IRON UPTAKE IN HYDROPONICALLY GROWN MAIZE

ABSTRACT

Humic acids have been shown to improve nutrient availability, particularly iron, to plants in hydroponics. This study was done to see what affects QH 6.6% humic acid from Horizon Ag Products (Modesto, CA), have on Fe availability in hydroponically grown Maize. This humic acid did not improve iron uptake compared the commercially available iron chelate controls. QH 6.6% humic acid appeared to coat roots in the current system this may have partially caused the lack of iron uptake.

In a second experiment a fulvic acid was used. Fulvic acids are more water soluble which should keep them from falling out of solution or binding to root surfaces. In this study Amber fulvic acid from Horizon Ag Products was used in hydroponics with maize plants. The fulvic acid appeared to increase iron availability and uptake over all commercially available chelates. There were problems with the nutrient solution in this study so it should be repeated to confirm the results.

INTRODUCTION

Several researchers have used humic and fulvic acids in hydroponics and observed increased nutrient uptake. Adani et al. (1998) looked at tomato plant response to two commercially available humic acids, one peat and one leonardite derived. Both products were applied at 20 and 50 mg/liter in a hydroponic system. They found that the peat product improved root fresh and dry mass at both concentrations, but did not significantly affect shoot growth when compared to a non humic acid control. The leonardite product improved shoot and root fresh and dry masses at 50 mg/liter, but only shoot dry mass at 20 mg/liter.

In addition they analyzed both roots and shoots for N, K, Ca, Mg, P, Fe, and Cu. In the shoots, the peat product improved Cu uptake at 20 mg/liter, and the leonardite product improved P concentration at 50 mg/liter. In the roots the peat compound increased N, Ca, P, Fe, and Cu at the lower concentration but only P and Fe at the higher concentration. The leonardite compound increased P and Fe at 50 mg/liter and Fe and 20 mg/liter.

Rauthan and Schnitzer (1981) found that a soil fulvic acid at 100 and 300 ppm increased root length and mass, shoot height and mass, number of flowers and N, P, K, Ca, Mg, Cu, Fe, and Zn concentrations in cucumber shoots.

However, Cooper et al. (1998) did not see nearly as beneficial results. In fact four of five tested humic acids did not improve root length in a hydroponic system, the one treatment that did was a leonardite derived product. All the products increased potassium uptake and one increased iron uptake. There was no difference for other nutrients.

MATERIALS AND METHODS

Experiment 1

120 Maize seeds (cv. Bodacious) were started in a germination box. Seven days later the seeds were moved to a vertical germination position where they were in paper towels and placed in a beaker that had a small amount of water in the bottom, they were kept wet by daily refills of the beaker. A large amount of fungus was growing on the

seeds when they were moved to paper towels, so a 0.1mmol solution of Subdue (a fungicide) was added to the water.

The experiment began when a majority of the plants had sent out their first leaf; this occurred fourteen days after the seeds were started. The experiment consisted of eight controls and one humic acid, done with two replications. The controls were (the first three were maintained at pH = 5.5 the others had no pH control):

No iron

No chelate

HEDTA all maintained at a pH of 5.5

No iron

No chelate

HEDTA

EDDHA

DTPA with no pH control.

QH 6.6% was the humic acid investigated in this experiment

HEDTA, EDDHA, and DTPA are standard iron chelating agents used in research. Each replicate was set up randomly, the nine treatments being set up in a three row by three-column pattern. Each plant was placed individually into a two liter brown plastic bottle. The bottles were filled with the nutrient solution in Table A1.

All treatments that had iron had10 μM $FeCl_3$ in their nutrient solution. The plants were held in place by soft foam plugs, this allowed the plants to grow and expand without impediment. Air was continuously bubbled into each bottle (Figure A1).

TABLE A1. Nutrient solution used in hydroponic experiment one.

Salt	ml of stock/100 L	Final Concentration
$Ca(NO_3)_2$	100	1 mM
$K(NO_3)$	50	1 mM
KH_2PO_4	100	0.5 mM
$MgSO_4$	50	0.5 mM
K_2SiO_3	100	0.1 mM
$MnCl_2$	5	3 µM
$ZnCl_2$	30	6 µM
H_3BO_3	5	2 µM
$CuCl_2$	15	3 µM
Na_2MoO_4	10	0.1 µM

pH was adjusted to 5.6 using 1 M HNO_3 as needed.

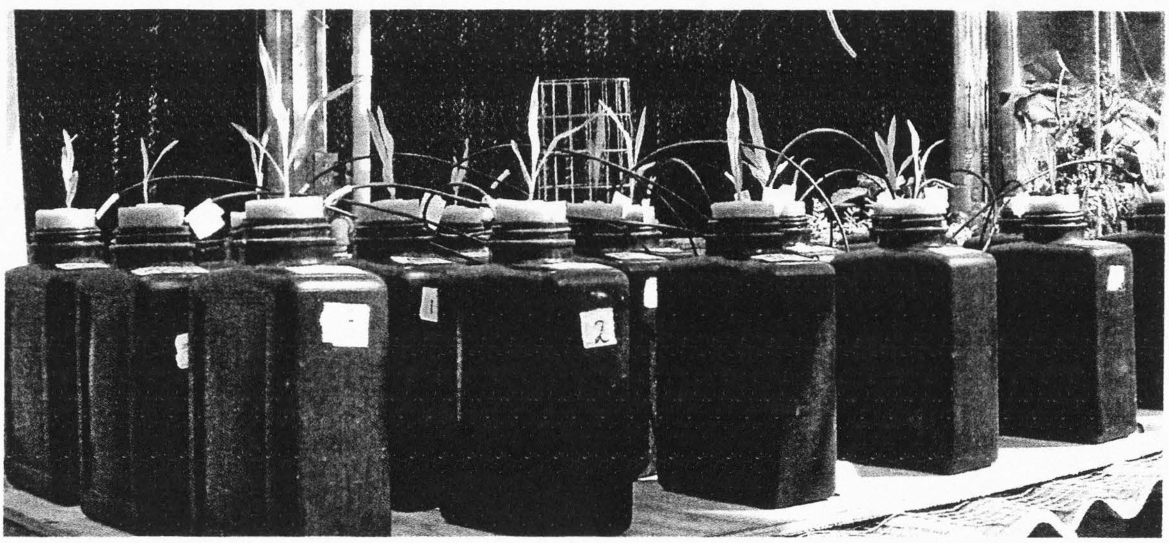

FIGURE A1. Hydroponic study set up using brown Nalgene bottles with foam in the mouths to hold the plants. Black tubes are for air bubbled into nutrient solution.

Once again the fungicide Subdue (0.5mmol solution) was used when the plants were moved into the bottles. Despite the use of fungicide, fungal growth continued to be a problem throughout the study.

The plants that were used were chosen based on similar root size. The rest of the plants were maintained in the vertical germination position, but were given nutrient solution to keep them similar to the plants used in the experiment in case a plant needed to be changed. Within two days about five plants were replaced with healthier plants, there were no more substitutions after this.

The treatments were checked daily for fluid level and twice a week for pH. When needed the appropriate solution was added, and pH was adjusted using 0.5 molar nitric acid. PH was measured using a HANNA mobile pH probe; it was calibrated on each day of use. On day 26 all the plants looked bad, the cause was the nutrient solutions in the bottles was 30-35^{0}C so the bottles were wrapped in tinfoil to keep the root zones cooler, this appeared to help.

Leaf chlorophyll content was measured using a Minolta SPAD-502 model chlorophyll meter. SPAD measurements were taken five times throughout the experiment, on days 21, 24, 28, 31, and 35. SPAD meters measure chlorophyll by shining a light on the leaf and measuring the amount of red and far-red light that passes through the leaf. Chlorophyll adsorbs red light and it lets most far-red light pass so the smaller the red: far-red ratio measured through a leaf the more red light that was absorbed by chlorophyll. The amount of light absorbed determines how much chlorophyll is in the leaf. Chlorophyll in a leaf is a good indicator of the amount of iron in the leaf.

Plants were harvested on the 36th day and were split into stem and root sections. Each section was placed in a paper sack and dried for two days. Plant dry weight was taken at the end of the drying period. All statistical calculations were done using Sigmastat 2.03 ANOVA two-way comparison tests.

Experiment 2

Maize (cv. Bodacious) seeds were germinated in the laboratory using slant boards. When large enough (8 to 13 days later), seedlings were transplanted into a tub containing the same nutrient solution used in experiment one. After an additional 5 to 10 days, 18 plants of similar size were transplanted into individual 2-L brown plastic Nalgene bottles containing the nutrient solution in Table A2.

TABLE A2. Nutrient solution used in hydroponic experiment two.

Salt	ml of stock/100 L	Final Concentration
$Ca(NO_3)_2$	400	4 mM
$K(NO_3)$	200	4 mM
KH_2PO_4	100	0.5 mM
$MgSO_4$	50	0.5 mM
K_2SiO_3	100	0.1 mM
$MnCl_2$	10	6 μM
$ZnCl_2$	15	3 μM
H_3BO_3	2.5	1 μM
$CuCl_2$	10	2 μM
Na_2MoO_4	5	0.05 μM

pH was adjusted to 5.6 using 1 M HNO_3 as needed.

Solutions in the individual bottles differed only in the presence or absence of iron (treatments with iron had 2.5 μM $FeCl_3$) and in the type of iron chelate, as indicated by the treatment. The bottles were wrapped in aluminum foil to keep the root zone from getting too hot. Hydroponic solution was checked daily and replenished as needed.

The experiment consisted of the same controls as before with "Amber" fulvic acid replacing QH 6.6% humic acid. There were two replicates; each replicate was set up in a randomized design.

The treatments ran for seventeen days. During the experiment pH and SPAD measurements were taken three times a week. The pH was adjusted using 0.5M nitric acid and MES buffer was used to help maintain pH. The pH was checked daily during the last week of the experiment.

The plants were weighed at the beginning of the treatments after one week and at the end of the experiment. Plants were weighed during the experiment by taking them out of solution and blotting the roots dry with absorbent paper then weighing them, they were immediately returned to solution.

Plants were harvested on the 35th day, and were separated into root and shoot sections. Fresh mass and a dry mass were taken for each section.

RESULTS

Experiment 1

Many of the plants started showing detrimental effects from the treatments within a few days after the experiment started, in fact two had already died by the first SPAD measurements seven days into the experiment (Figure A2).

Throughout the study SPAD measurements tended to decrease for all treatments except the HEDTA at pH 5.5 treatments (Figure A3). One treatment that had no iron available to it maintained a relatively high SPAD value and it survived until the end of the study. All other plants that survived the entire experiment had an iron chelater; these plants also maintained the highest SPAD values. Most of the SPAD values were low when compared to a typical crop plant. Under normal conditions healthy plants should have SPAD values between 30 and 40, but in this study only four values got above 25.

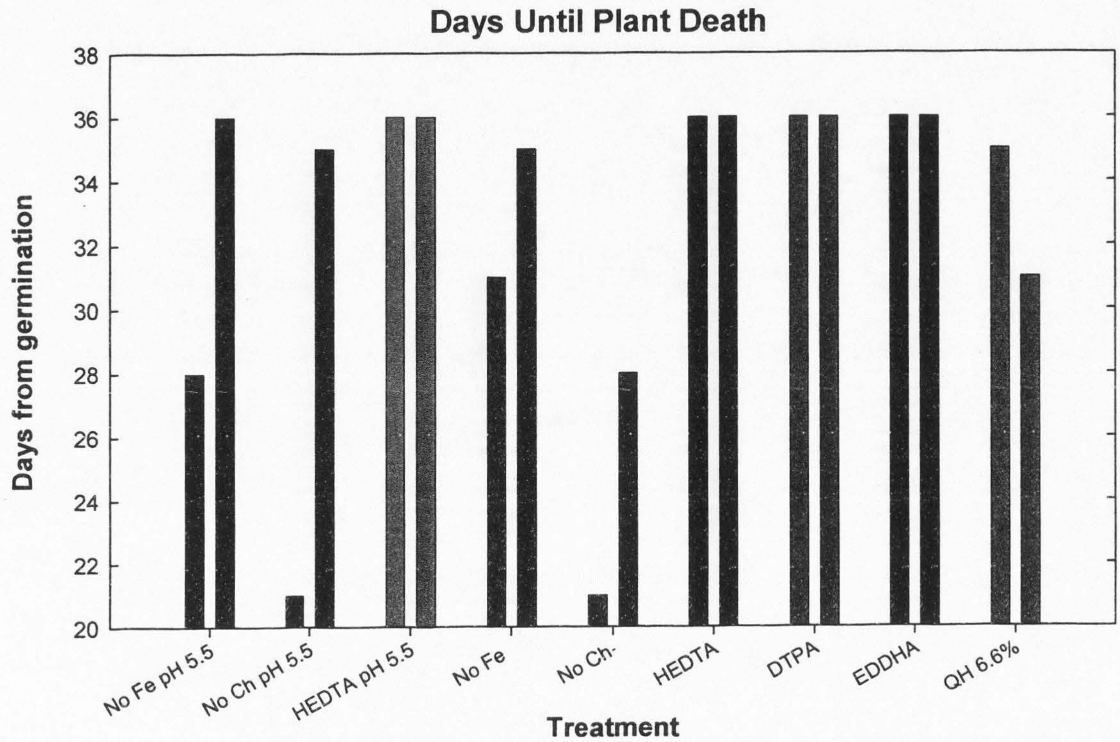

FIGURE A2. Plant survival time for each replicate, measured from the start of seed germination. The first replicate is on the left for each treatment. All plants were harvested on day 36.

The stem weight from the HEDTA treatment at pH 5.5 was not statistically different (p=0.051 for no chelate, and HA treatments) from the other treatments. HEDTA at pH 5.5 treatments root weight is significantly different (p=0.019 to 0.025) than all of the other treatments root weights except for the HEDTA treatment (p=0.864). This same result is repeated in the plant total weight analysis, with p values of 0.983 for the HEDTA treatment and 0.041 to 0.047 for all other treatments (Figure A4).

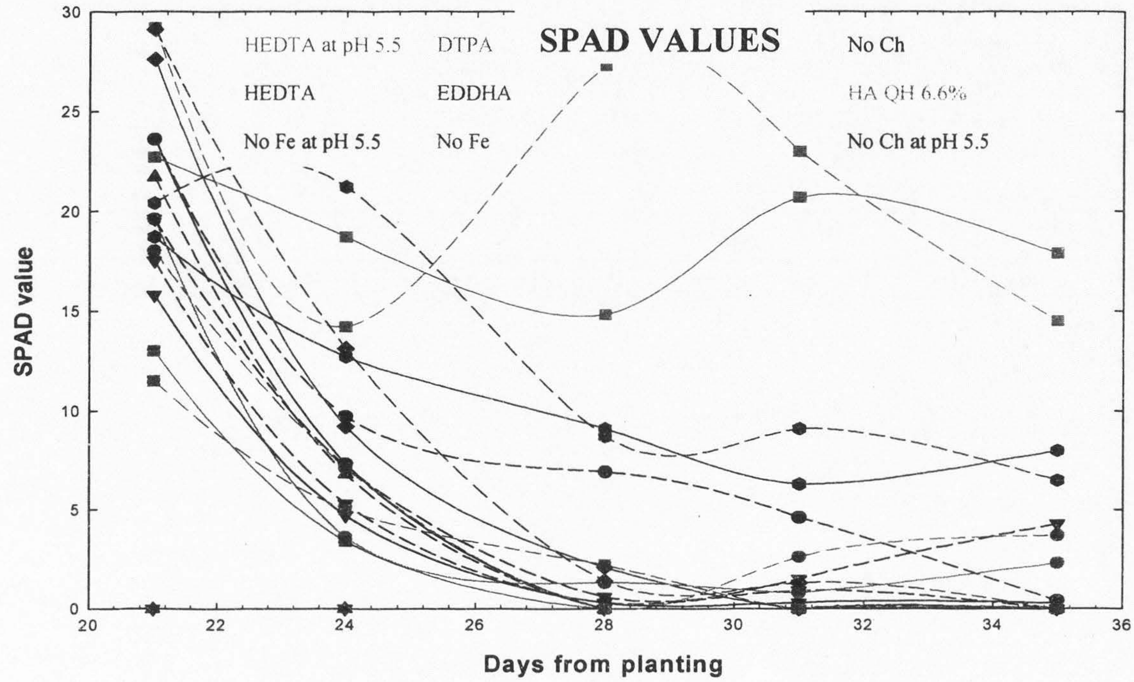

FIGURE A3. Shows the changing SPAD values throughout the experiment for each plant.

There was no difference between the treatments that were without iron and chelate, ones with iron without chelate and ones with iron and chelate on stem, root or total mass, except for HEDTA treatment.

Both HEDTA treatments were significantly different from the other treatments for stem mass to root mass ratio (p=0.004 to 0.042) (Figure A5), but the other treatments were not significantly different from each other (p=0.981). Typically a healthy plant has a one to one relationship between stem and root surface area. Because roots are not as thick as stems, plants usually have more stem mass than root mass so we would expect to see a ratio larger than one like the HEDTA treatments. The fact that all the other treatments have a ratio near one shows that they were spending their energy to grow root systems, this is typical of plants under stress. Presumably in this study it is iron stress that has affected the plants.

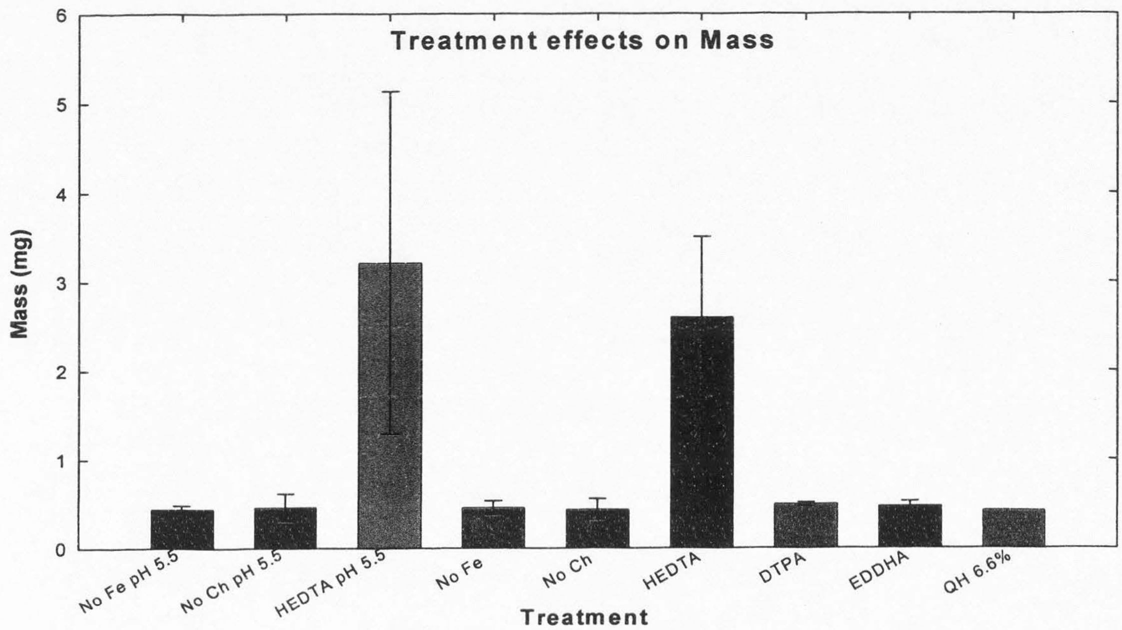

FIGURE A4. Average total weights from each treatment with the error bars showing one standard deviation.

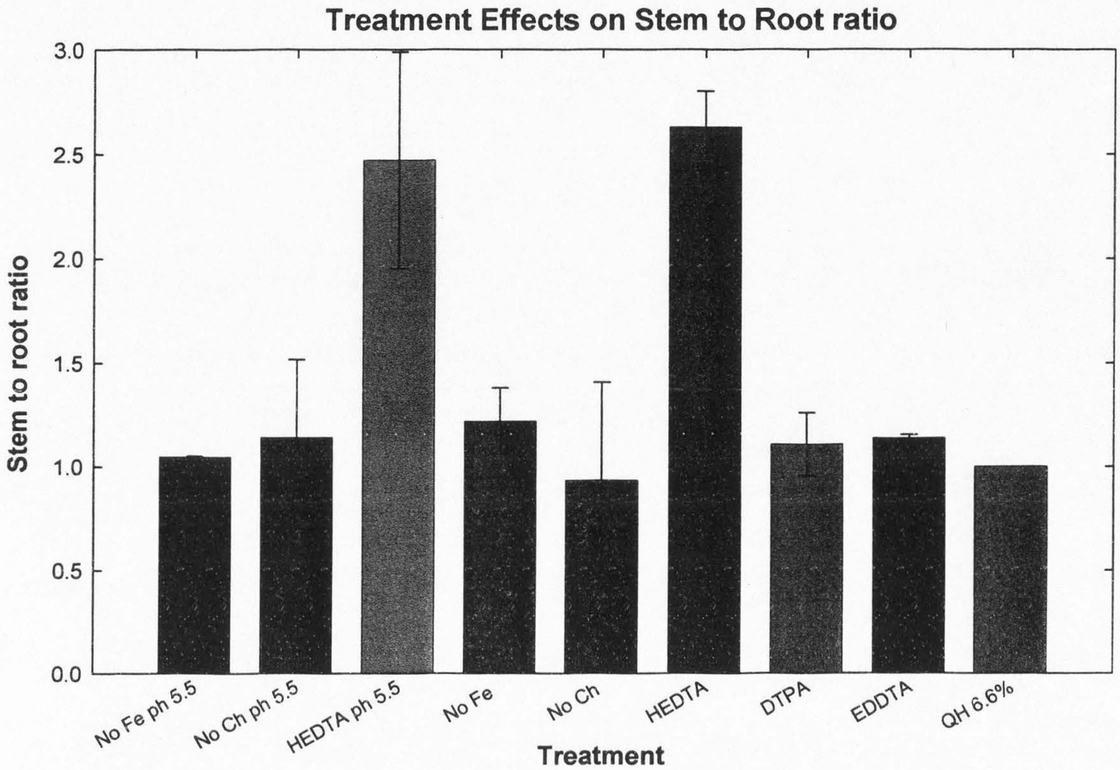

FIGURE A5. Stem mass to root mass ratio for each treatment with standard deviations bars.

Experiment 2

The switch in nutrient solutions at the beginning of the experiment caused severe iron stress and chlorosis, because the second nutrient solution has four times less available iron. Despite the observed iron chlorosis, differences in final plant mass indicate that there were some treatment effects (Table A3).

TABLE A3. Treatment effects on plant mass. Values shown are the mean of two replicates.

pH controlled at 5.5	Mean Wt. (g)
No Fe+	8.5
Fe+, No Chelate	7.1
HEDTA	28.9

No pH control	
No Fe+	6.1
Fe+, No Chelate	7.7
HEDTA	22.2
DTPA	9.4
EDDHA	12.2
Amber FA	35.2

DISCUSSION

Experiment one had problems from the start. The fungus that grew on the plants was a persistent problem throughout the entire experiment except on the HEDTA treated plants, the DTPA plants and one of the EDDHA plants. The fungus may have caused some of the differences seen in the experiment. The stronger plants were able to overcome the fungus, while the other plants may have died quicker with the fungus sapping energy from them. Another problem was a lack of good healthy plants after germination making it impossible to select a uniform group for the experiment. As a result the plants were selected on whether or not they looked like they could survive through the experiment. In addition, it is unknown what affect the high temperatures of the root zone may have had.

The experiment was designed to have several of the treatments do well. Because all the plants showed early drops in health it is believed that the experiment was started too early in their life cycle, before they could cope with the stress levels.

The HEDTA treatments in experiment one did the best and that was expected, because the other chelators bind iron so tightly plants can not get the iron from them, until the pH get into the high sevens. The pH for these plants never got that high (data not shown). The one plant from the no iron treatment that kept a high SPAD value and survived the whole experiment was a very small plant (even smaller than the other no iron plants that died) it appeared to be in a state of no growth.

In experiment two the HEDTA treatment's stem mass was significantly different than the other treatments. The p value for the test was 0.51, which is right on the border of being significant. If this study were repeated with improved techniques the HEDTA stem mass should be significant. The reason it was close to significance in the present test may be due to the large standard deviation of the HEDTA treatments. The reason no difference in treatment types (i.e. no iron, no chelate, some chelate) was seen is that the only chelate that significantly helped the plants was HEDTA. It is interesting to note that most of the plants had a stem to root ratio close to one because this is typical of stressed plants. These plants were given all nutrients needed for growth except iron, so iron stress probably caused these results.

Having a chelator of any kind, except the humic acid, did help the plants survive in experiment one. From similar reports it was expected that the humic acid would have a similar effect. Information collected near the end of the experiment suggests this humic acid coated the roots thus making it impossible for the plants to get nutrients. This would explain the lack of iron uptake and plant death. All SPAD values throughout the study were lower than normal, and this could be a result of starting early in the plant's life and the persistent fungus.

The expected outcome of experiment one was a measurable difference between the humic acid treatment and the no iron and no chelate treatments. Also that the humic acid treatment would fall somewhere in between the different chelate treatments. Neither of these results was seen. This may have been caused by the problems mentioned before, but the most probable cause of these results is the fact that the humic acid coated plant roots, eliminating nutrient uptake.

HEDTA still appears to be the best iron chelator of the standard commercially available chelates as expected. "Amber" fulvic acid appears to have had even a larger effect than HEDTA, meaning it is capable of chelating iron. It appears that fulvic acids have greater effects in hydroponics then humic acids do, this agrees with other reported findings.

Fulvic acids appear to be effective iron chelators in hydroponics systems. Additional research needs to be done with a slower transition from one nutrient level to the next. This same study should be run in sand or soil to see if Amber fulvic acid could be used to reduce iron chlorosis in agricultural and gardening conditions.

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APPENDIX B. GREEN FRUIT HARVESTED IN DRY GRANULAR STUDIES (FROM CHAPTER 3)

Experiment 1

The green fruit harvested at the end of the experiment showed that Luscar and Aldrich treatments produced more green tomato fresh mass than the other treatments. ND leonardite produced the least (Figure B1A; Tables B1A, B1B).

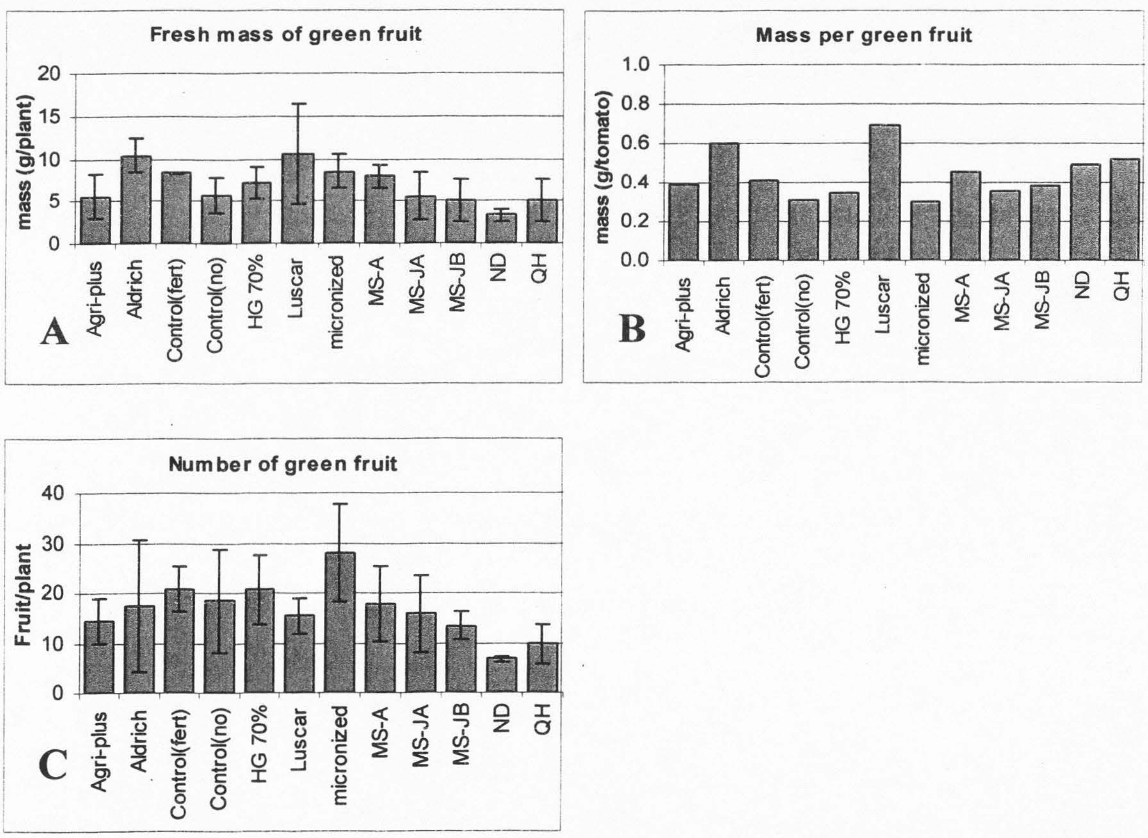


FIGURE B1. A) Fresh mass of green fruit per plant B) fresh mass of each green fruit and C) number of green fruit per plant.

Luscar, Aldrich, ND leonardite and QH treatments all had greater average fresh mass per fruit than the controls (Figure B1B). Only the Micronized treatment had a greater number of fruit than the controls, while ND leonardite and QH had less fruit than the control (Figure B1C). None of the differences seen in average mass per fruit or the number of fruit was statistically significant (Table B1A).

TABLE B1. A) Statistical analysis of the green fruit data, B) mean separation of the treatments for fresh mass of fruit.

A	DF	F Value	Pr > F	Coeff Var
number of fruit	11	1.76	0.12	43.6
fresh mass	11	2.28	0.04*	37.4
mass/fruit	11	1.19	0.35	54.6
% fresh	11	0.59	0.82	9.3

B			
Treatment	**Fresh Mass**		**% of control**
Luscar	10.5	a	126
Aldrich	10.4	a	124
Micronized	8.5	ab	101
Control (w/fert)	8.4	ab	100
MS-A	8.0	ab	95
HG 70%	7.2	abc	86
Control (w/o fert)	5.7	bc	68
Agri-plus	5.6	bc	67
MS-JA	5.6	bc	66
MS-JB	5.0	bc	60
QH	5.0	bc	60
ND	3.3	c	39
LSD	4.37		

Experiment 2

The green fruit harvest with the second experiment of the dry granular product studies. There were no significant differences between the green fruit number and mass per plant. Nor was there any difference in mass per fruit or percent fresh mass (Table B2).

	Green fruit data			
	fruit /plant	**mass /plant**	**mass /fruit**	**% fresh mass**
HG 70%	3.75	9.57	4.09	7.73
MS-A	2.50	10.44	3.62	8.34
ND	2.25	13.30	4.81	7.93
Blend	1.67	6.77	2.51	8.07
MS-JA	1.50	6.30	2.02	8.80
MS-B	1.50	8.60	2.75	8.16
Control	1.38	4.82	3.52	7.67
Agri-plus	0.25	4.20	4.20	8.10
F Value	1.03	1.71	0.63	0.56
Pr > F	0.43	0.19	0.73	0.78
Coeff Var	113.0	45.3	52.9	9.82

TABLE B2. Green fruit harvest data with statistical analysis.

APPENDIX C. (ADDITIONAL RESEARCH) FOLIAR APPLICATION OF ORGANIC AMENDMENTS TO SOYBEANS

ABSTRACT

It has been suggested that foliar applied humic substances are possibly the only application method that will be economically feasible to get the desired beneficial results on plant growth. This study looked at the effects of five commercially available fulvic acid foliar sprays on soybean growth in a green house environment. The treatments were applied at 100 ml/liter. There were also three root zone treatments with one third of the plants in each. The root zone treatments were high pH (pH 8) stress, low nutrient and no stress. There were no significant differences on plant mass, time to flowering, leaf chlorophyll content and leaf to stem ratio in any of the root zone environments.

INTRODUCTION

A little research has been done on using humic substances as foliar sprays. Lee and Bartlett (1976) did not get significant yield increases when they used a 20 ppm foliar spray humic acid on algae.

Other researchers have seen beneficial effects. Chen and Aviad (1990) report that a foliar applied fulvic acid increased leaf chlorophyll levels, and helped the roots get phosphorus. Cooper et al. (1998) applied 4 foliar products on creeping bent grass in a quartz sand system at rates of 100, 200 and 400 mg/liter. The rate of application had no effect on plant growth. A soil derived product increased root mass at depths from 0-10 cm and >20 cm and a leonardite product increased root mass in depths >20 cm. Neither improved overall root length. In their study the leonardite extract increased P and K in the roots. The peat and soil extracts also increased phosphorus in the roots.

Most humic materials have been extensively studied in hydroponics with a few done in sand or soil. Foliar applied humic materials are the exception with large percentage of the research done in the field. Zhang et al. (2003) found that a seaweed extract applied with a humic acid as a spray reduced the damage done to Kentucky bluegrass sod by heat during transit and improved transplant rooting. Brownell et al. (1987) found two leonardite derived humic compounds significantly increased yield in tomatoes, cotton and vineyard grapes. These compounds increased flowering, and seed respiration when used together.

Further research on leonardite derived humic compounds found improved shoot growth and promoted uptake of K, B, Mg, Ca, and Fe in leaves of field grown olive trees (Fernandez-Escobar et al., 1996).

One of the most interesting things reported about foliar applied fulvic acids is that they may be able to reduce draught stress and damage. Chen and Aviad (1990) report foliar applied fulvic acid reduced stomatal conductance in pot grown wheat. It also helped plants maintain stomatal conductance throughout a drying cycle, and reduced yield loss caused by water stress from 30% loss to 3%.

Interestingly enough Chen and Aviad (1990) also report results indicated that fulvic acids were more beneficial to plant growth than humic acids when supplied foliarly. This is opposite of observations made on soil structure (see Chapter 1).

MATERIALS AND METHODS

The potential beneficial effects of foliar sprays of 5 organic amendments were evaluated using soybean plants (cv. Hoyt). The study included 54 plants in a 3 by 6 factorial experiment with the 5 foliar sprays and a control. The foliar sprays were prepared by at a concentration of 100 ml/liter. The treatments were:

F-6000

Aqua FA

Organic FA

VK FA

Amber FA

Control with no foliar spray

There were also 3 root-zone environments designed to compare the foliar treatments in low, medium, and high nutrient stress conditions. The plants were grown using coconut coir media in 12.7 cm (5 inch) pots in a greenhouse with supplemental lighting from high pressure sodium lamps. The day night cycle was 16/8 hours. There were 3 replicate pots of each foliar treatment in each root-zone environment. The root-zone treatments were:

1) high pH (pH 8)

2) low nutrient

3) unstressed control.

Seventy-five pots were initially started with 54 being selected by their uniformity for use in the study (Figure C1).

FIGURE C1. Soybeans grown in coconut coir, treated with foliar applied fulvic acids.

One replicate of each treatment was harvested on the 26th day of the experiment and had been sprayed twice at nine day intervals. This replicate was harvested to evaluate initial results, and to make more room for the remaining plants. The smallest plant from each treatment was selected for the initial harvest.

The remaining pH 8 plants were harvested on 35th day of the experiment. They had been sprayed three times at nine day intervals. This harvest was done to increase room for the remaining plants. All remaining plants were harvested on the 44th or the 49th day of the experiment and had been sprayed five times with the foliar solutions.

Several of the low nutrient plants began to flower on the 42nd day of the experiment, and some of the none-stressed plants flowered on the 45th day.

Each root-zone environment (no stress control, pH 8 stress, and low nutrient stress) was analyzed separately. The individual harvests were combined by normalizing the data to the final harvest. This was done by a calculating a normalization factor for each root zone environment. The factors were calculated by averaging the controls from the last harvests then dividing by the control in the first harvest. The normalization factors were then multiplied to the appropriate plants from the first harvest, and then they were combined with the other data.

RESULTS

There was no significant difference among treatments in the dry mass (Figure C2), or percent fresh mass (Figure C3). Some treatments percent fresh mass may be significantly less than the control in the low nutrient stress (Figure C3). There was also no significant in the chlorophyll content of the leaves; the time to flowering; or in the leaf to stem ratio (data not shown).

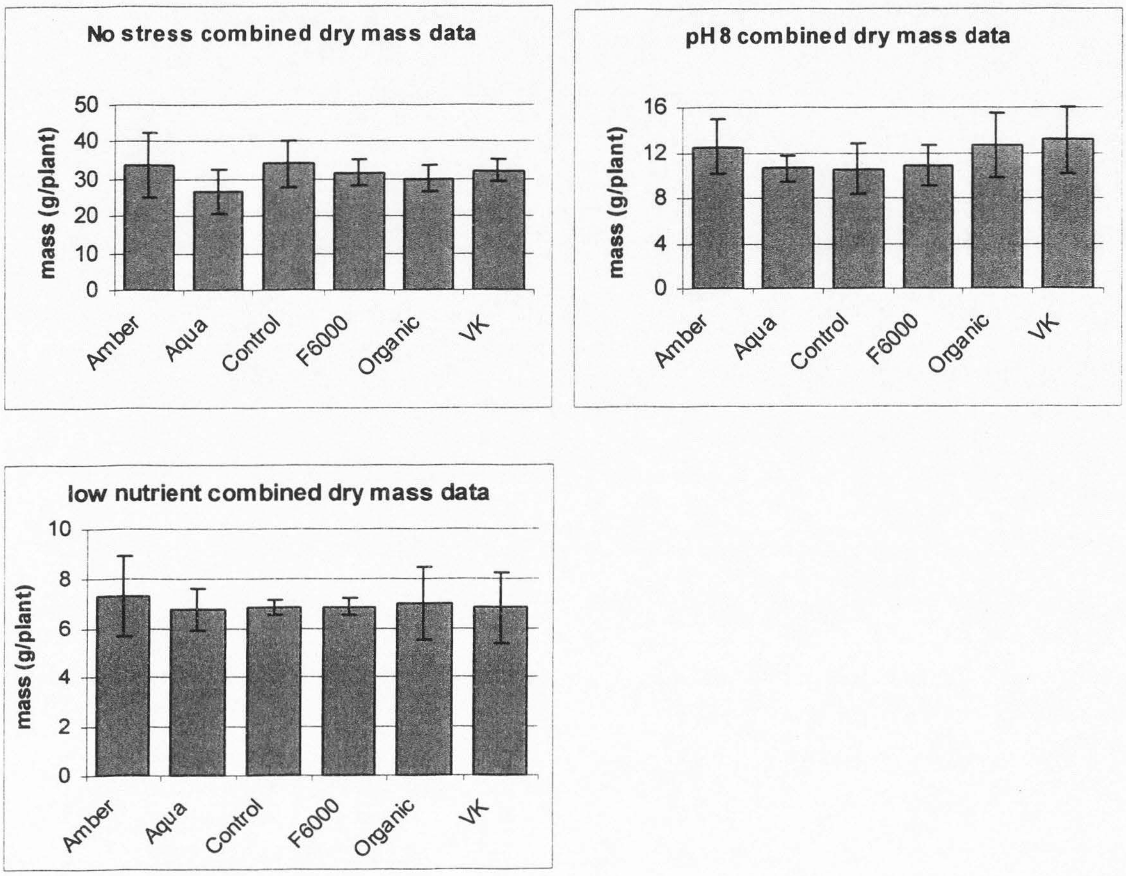
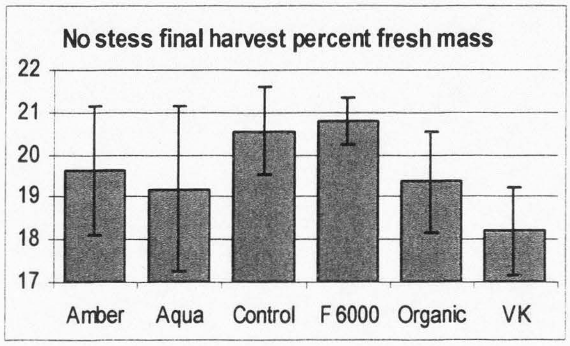


FIGURE C2. The combined dry mass data from each of the three root-zone environments.



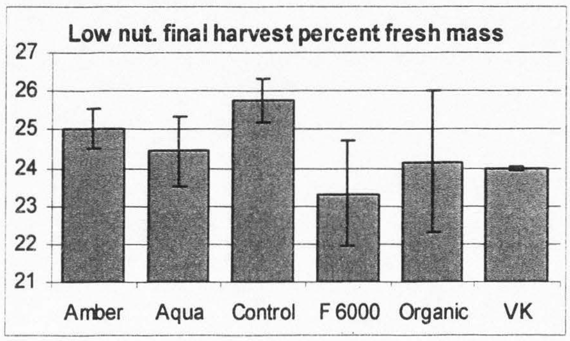


FIGURE C3. Percent fresh mass for no stress and low nutrient root-zone treatments. Percent Fresh mass was not measured in the pH 8 treatment.

DISCUSSION

The lack of treatment effects in this experiment does not suggest that further testing with soybeans isn't warranted. The largest beneficial effect of foliar sprays would likely occur when the plants are not getting all the nutrients they need through the roots. Although two levels of nutrient stress were studied, it is possible that nutrient elements supplied by the organic amendments were not the ones limiting growth.

Future studies with foliar sprays might use different levels of slow release fertilizer to more precisely stress the plants. Tests with another crop species might also be conducted. The Aqua treatment was the least beneficial, and it could be left out of future studies.

The plants did not grow in coir as well as they normally do in a controlled green house environment. Further research on how coir effects plant growth has been carried out in our lab since the conclusion of this study. The results are inconclusive at this time, but many sources of coir do appear to have adverse effects on plant growth. In future work a change from coconut coir to sand or soil would probably be wise and could result in noticeable treatment affects. This change of substrate would be more like the cited papers were many foliar studies have been done in field conditions (i.e. soil).

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APPENDIX D. (ADDITIONAL RESEARCH) LETTUCE GROWN IN FLORIDA MUCK SOILS: EFFECTS OF AN ORGANIC AMENDMENT 'HYDRA-HUME'

ABSTRACT

The effects of Hydra-Hume on lettuce growth were evaluated in a greenhouse study in two types of muck soils from Florida. Plants in the Pahokee soil were statistically larger than in the Torrey soil. There were three treatments: Hydra-Hume alone, fertilizer alone, and Hydra-Hume with fertilizer. The effects of fertilizer were large and significant for all measured parameters. The Hydra-Hume with fertilizer treatment, compared to fertilizer alone, was not statistically significant at the 5% probability level. However, the addition of Hydra-Hume resulted in an 8.3% increase in lettuce fresh mass on the Pahokee soil ($p=0.20$), and a 46% increase in root vigor on the Torrey soil ($p=0.14$).

INTRODUCTION

The effects of humic substances applied to a soil with high organic matter content would presumably be small or insignificant. There are no known studies that have attempted a similar undertaking.

MATERIALS AND METHODS

Leaf Lettuce (cv. Grand Rapids) was grown in 7.6 cm (3") diameter columns made of PVC pipe cut to a length of 46 cm (18") with a PVC cap on the end. A drain hole in the middle of the cap was covered with two layers of 16-mesh screen to keep soil in the column (Figure D1).

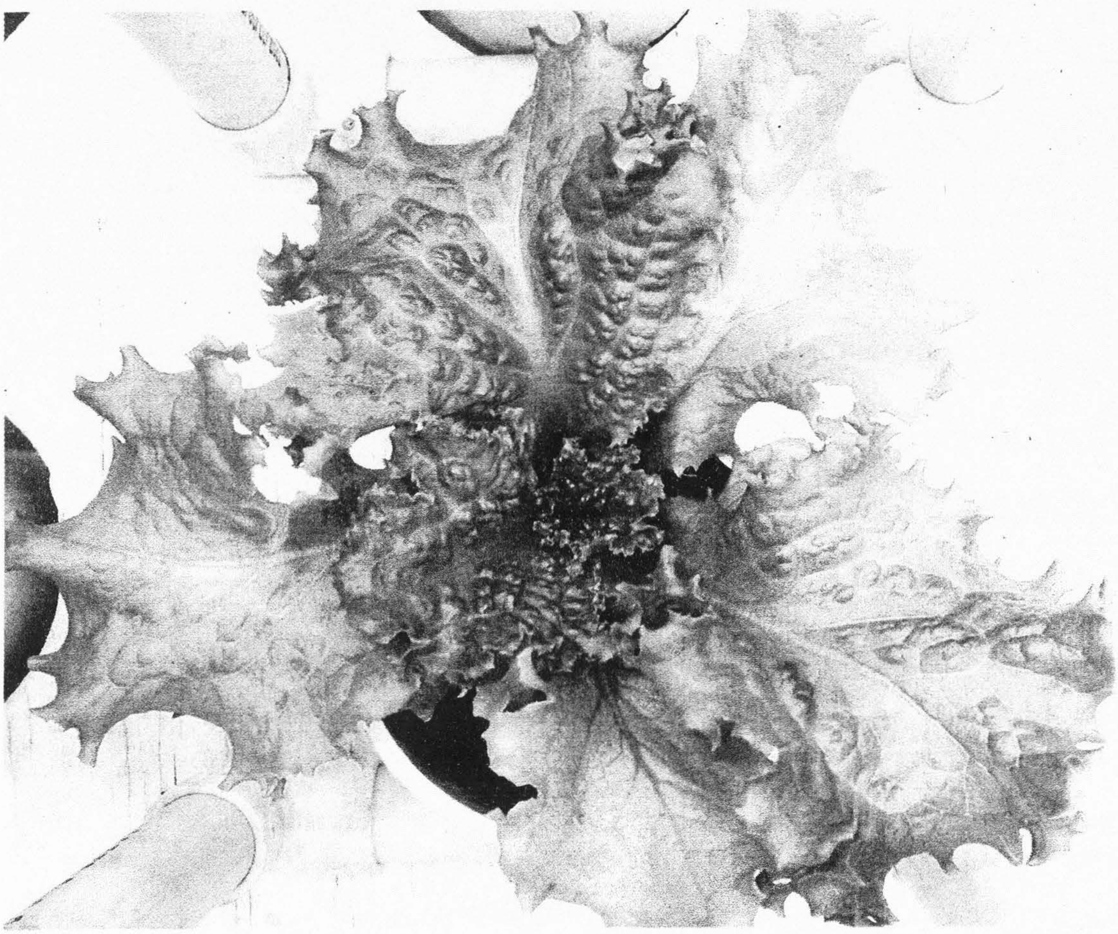

FIGURE D1. Lettuce grown in Florida muck soil treated with Hydra-Hume organic product.

Two muck soils, Torrey and Pahokee, from Florida were placed in the PVC columns and soil analysis was done on both (Table D1). Small amounts of both soils were pre-mixed with Hydra-Hume organic product at 80 lbs. per acre and this pre-treated soil was placed on the top 5 cm (2 inches) of each column.

TABLE D1. Soil characteristics for Pahokee and Torrey muck soils from Florida.

	Pahokee	Torrey	normal range
Texture	Loam	Loam	
pH	7.4	6.9	6.1-8.4
Salinity-EC	1.1	0.9	1.8-4.0
P mg/kg	11.4	4.5	19-60
K	129	164	125-400
N mg/kg (Nitrate-Nitrogen)	80.8	17.5	>25

There were three treatments:

Fertilizer only (without Hydra-Hume)

Hydra-Hume without Fertilizer

Hydra-Hume with Fertilizer

Fertilizer was applied at 60-60-120 N-P-K at a rate of 61 lbs/acre before planting and was mixed into the top two inches of the columns. This fertilizer mixture was made using chemical grade ammonium nitrate (NH_4NO_3), potassium phosphate (K_2HPO_4) and potassium phosphate monobasic (KH_2PO_4). The columns were arranged in a randomized complete block design.

The plants were started by soaking the soil with tap water then placing the seeds on top of the soil. The seeds were covered by a thin layer of vermiculite to keep them moist and improve germination. Approximately 5-10 seeds were started in each column, and thinned to one uniform plant over the two weeks following emergence. The plants were grown in a greenhouse with supplemental light from high pressure sodium lamps. They were grown with a day/night cycle of 16/8 hours. All columns were watered with tap water every two to three days. The columns were leached weekly.

Digital pictures were taken of each plant each week. Pixel counts were done (using Adobe Photoshop 6.0) to calculate relative growth rates. This method of measuring plant growth was developed in our laboratory and described in a paper by Klassen et al. (2003).

Plants were harvested on the 52nd day of the experiment. Fresh and dry weights were taken. The roots were scored on a scale from 1 to 5, based on how deep they grew in the columns as well as how thoroughly they penetrated and used the entire soil column. All statistical analyses were done by an ANOVA test using SAS software (Version 9.0).

RESULTS

Pixel count data showed that the plants were significantly larger in the Pahokee soil on February 18th and from March 4th through the end of the experiment (Table D2).

TABLE D2. Pixel count data (measured in kilopixels) taken each week during the experiment.

	4-Feb	11-Feb	18-Feb	25-Feb	4-Mar	11-Mar
Pahokee	12.2 a	71.1 a	250 a	293 a	988 a	1517 a
Torrey	13.5 a	70.9 a	211 b	252 a	770 b	1283 b
HH & Fertilizer	15.8 a	96.4 a	332 a	385 a	1200 a	1883 a
Fertilizer only	14.5 a	95.9 a	313 a	376 a	1195 a	1872 a
Hydra-Hume only	8.3 b	20.6 b	45 b	56 b	241 b	447 b

* Treatments with different letters are significantly different at the $p = 0.05$ level.

This was also seen in the harvest data with the Pahokee soil having greater fresh and dry masses (Table D3). Root vigor was also greater in the Pahokee soil (Table D3).

TABLE D3. Plant harvest data including fresh and dry mass, and root growth. There was no significant difference in % dry mass.

	Fresh mass (g)	Dry mass (g)	% Dry mass	Root vigor
Pahokee	15.2 a	1.11 a	7.14	2.71 a
Torrey	9.5 b	0.64 b	6.73	1.75 b
HH & Fertilizer	17.2 a	1.19 a	7.08	2.88 a
Fertilizer only	16.5 a	1.19 a	6.92	2.56 a
Hydra-Hume only	3.4 b	0.23 b	6.80	1.25 b

* Treatments with different letters are significantly different at the $p = 0.05$ level.

There was no difference between the fertilizer only and the fertilizer plus Hydra-Hume treatments in any measured parameters, but both of these treatments were significantly larger than the Hydra-Hume only treatment in all measured data except for percent dry mass (Tables D2, D3, D4 & D5). The differences in plant growth using pixel counts are shown in Figure D2.

TABLE D4. F values, probability and coefficient of variation for the pixel count data in Table 1, includes soil and treatment interaction.

		F Value	Pr > F	Coeff Var
4-Feb	**Soil**	4.4	0.0503	12.2
	Treatment	53.0	<.0001	
	Soil*Treatment	0.3	0.7150	
11-Feb	**Soil**	0.0	0.9701	18.2
	Treatment	91.0	<.0001	
	Soil*Treatment	2.1	0.1560	
18-Feb	**Soil**	6.2	0.0233	16.9
	Treatment	136.0	<.0001	
	Soil*Treatment	4.8	0.0212	
25-Feb	**Soil**	4.1	0.0583	17.9
	Treatment	118.0	<.0001	
	Soil*Treatment	2.4	0.1174	
4-Mar	**Soil**	9.3	0.0069	20.0
	Treatment	79.0	<.0001	
	Soil*Treatment	4.2	0.0319	
11-Mar	**Soil**	9.7	0.0060	13.1
	Treatment	162.0	<.0001	
	Soil*Treatment	5.7	0.0120	

TABLE D5. F values, probability and coefficient of variation for harvest data in Table 2. includes soil and treatment interaction.

		F Value	Pr > F	Coeff Var
Fresh mass (g)	**Soil**	49.9	<.0001	16.0
	Treatment	123.8	<.0001	
	Soil*Treatment	18.8	<.0001	
Dry mass (g)	**Soil**	47.6	<.0001	19.1
	Treatment	89.4	<.0001	
	Soil*Treatment	16.2	<.0001	
% Dry mass	**Soil**	3.4	0.0826	8.01
	Treatment	0.5	0.6072	
	Soil*Treatment	1.2	0.3335	
Root vigor	**Soil**	12.3	0.0025	30.0
	Treatment	13.2	0.0003	
	Soil*Treatment	3.9	0.0383	

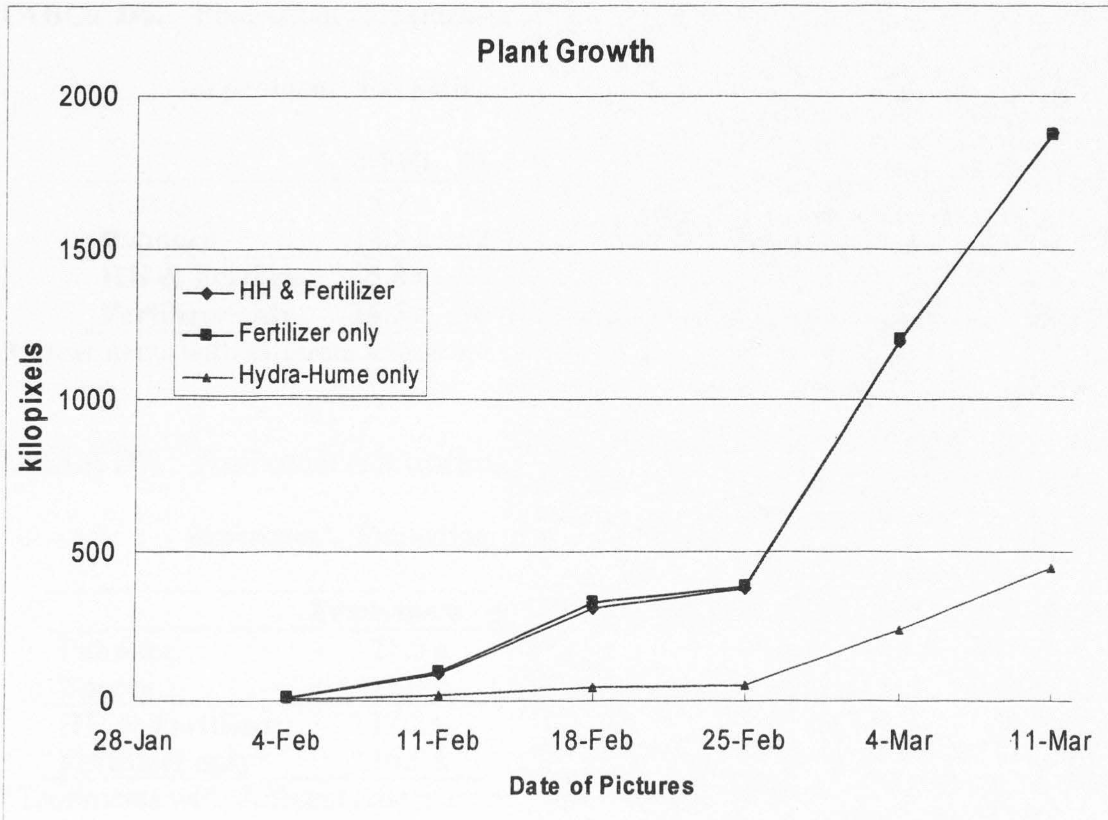

FIGURE D2. Plant growth for each treatment measured by pixel counts using digital images.

In an effort to associate small treatment effects with statistical significance, the unfertilized treatment was removed and the data reanalyzed. This analysis showed a more consistent affect on pixel counts between soils, with the significance difference starting on February 18th and continuing throughout the remainder of the experiment (Table D6). The difference between soils remained in the harvest date (Table D7). This analysis did not show any differences between the fertilizer only and the fertilizer plus Hydra-Hume treatments for any measured parameter (Tables D6 & D7). In addition, only the pixel count data on February 4th was close to significant between these treatments (Tables D8 & D9).

TABLE D6. Pixel count data (measured in kilopixels) taken each week during the experiment. Excluding the Hydra-Hume only treatment.

	4-Feb	11-Feb	18-Feb	25-Feb	4-Mar	11-Mar
Torrey	15.9 a	99.3 a	286 b	345 b	1015 b	1672 b
Pahokee	14.5 a	92.9 a	360 a	416 a	1380 a	2084 a
HH & Fertilizer	15.8 a	95.9 a	313 a	376 a	1195 a	1884 a
Fertilizer only	14.5 a	96.4 a	332 a	385 a	1200 a	1872 a

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE D7. Pixel count data (measured in kilopixels) taken each week during the experiment. Excluding the Hydra-Hume only treatment.

	Fresh mass (g)	Dry mass (g)	% Dry mass	Root vigor
Pahokee	21.5 a	1.56 a	7.27	3.44 a
Torrey	12.3 b	0.82 b	6.73	2.00 b
HH & Fertilizer	17.2 a	1.19 a	6.92	2.88 a
Fertilizer only	16.5 a	1.19 a	7.08	2.56 a

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE D8. F values, probability and coefficient of variation for the pixel count data in Table 5, includes soil and treatment interaction.

		F Value	Pr > F	Coeff Var
4-Feb	**Soil**	2.06	0.177	12.2
	Treatment	1.98	0.185	
	Soil*Treatment	0.50	0.495	
11-Feb	**Soil**	0.68	0.426	16.2
	Treatment	0.00	0.949	
	Soil*Treatment	0.65	0.436	
18-Feb	**Soil**	9.65	0.009	14.7
	Treatment	0.66	0.434	
	Soil*Treatment	0.24	0.633	
25-Feb	**Soil**	6.12	0.029	15.2
	Treatment	0.10	0.762	
	Soil*Treatment	0.01	0.944	
4-Mar	**Soil**	12.40	0.004	17.3
	Treatment	0.00	0.960	
	Soil*Treatment	0.07	0.801	
11-Mar	**Soil**	18.3	0.001	10.3
	Treatment	0.02	0.903	
	Soil*Treatment	0.15	0.705	

TABLE D9. F values, probability and coefficient of variation for harvest data in Table 6, includes soil and treatment interaction.

		F Value	Pr > F	Coeff Var
Fresh mass (g)	**Soil**	66.0	<.0001	13.4
	Treatment	0.4	0.5466	
	Soil*Treatment	0.7	0.4151	
Dry mass (g)	**Soil**	57.0	<.0001	16.4
	Treatment	0.0	0.9701	
	Soil*Treatment	0.0	0.9900	
% Dry mass	**Soil**	3.5	0.0863	8.3
	Treatment	0.3	0.5941	
	Soil*Treatment	1.6	0.2354	
Root vigor	**Soil**	18.0	0.0012	25.0
	Treatment	0.8	0.3767	
	Soil*Treatment	1.7	0.2230	

Analysis of pixel count data for
individual soils

There was a significant fertilizer effect in both soils throughout the study (Table D10 and D11). In addition there was a difference seen between the fertilizer only and the Hydra-Hume treatments in the Torrey soil, but this difference flip-flopped from Feb. 4th to Feb. 18th. Later in the experiment differences between these two treatments disappeared.

TABLE D10. Pixel count data (measured in kilopixels) taken each week during the experiment for Pahokee and Torrey muck soils.

Soil	Treatment	Feb. 4	Feb. 11	Feb. 18	Feb. 25	Mar. 4	Mar.11
Torrey	Fertilizer	14.9 b	103 a	302 a	351 a	1005 a	1685 a
	Hydra-Hume	9.0 c	14 b	60 c	67 b	278 b	508 b
	HH & Fertilizer	16.8 a	96 a	271 b	340 a	1026 a	1659 a
Pahokee	Fertilizer	14.2 a	90 a	363 a	420 a	1396 a	2059 a
	Hydra-Hume	7.6 b	27 b	31 b	45 b	204 b	386 b
	HH & Fertilizer	14.9 a	95 a	356 a	413 a	1364 a	2108 a

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE D11. Statistical analysis of pixel count data for each week in Pahokee and Torrey muck soils, all three treatments are included.

	--------------Pahokee----------------			----------------Torrey---------------		
Date	**F Value**	**Pr > F**	**Coeff Var**	**F Value**	**Pr > F**	**Coeff Var**
4-Feb	18.1	0.0007	15.6	49.7	<.0001	8.6
11-Feb	92.0	<.0001	11.1	36.2	<.0001	23.2
18-Feb	49.3	<.0001	21.6	680.0	<.0001	4.8
25-Feb	68.4	<.0001	17.7	49.4	<.0001	18.1
4-Mar	54.4	<.0001	18.6	26.2	0.0002	21.6
11-Mar	512.0	<.0001	5.7	30.1	0.0001	19.1

Analysis of pixel count data for the Fertilizer only and HH plus fertilizer treatments in individual soils

In an effort to associate small treatment effects with statistical significance, the unfertilized treatment was removed and the data reanalyzed. This removed all significant differences (Table D12), except for in the Torrey soil on Feb. 18th. The Torrey soil on Feb. 4th was still close to significance. Like in the previous section the better treatment was not consistent for both of these dates (Table D13).

TABLE D12. Statistical analysis of pixel count data for each week in Pahokee and Torrey muck soils, excluding the Hydra-Hume treatment.

	------------Pahokee--------------			----------------Torrey---------------		
Date	**F Value**	**Pr > F**	**Coeff Var**	**F Value**	**Pr > F**	**Coeff Var**
4-Feb	0.16	0.70	15.91	5.11	0.06	7.70
11-Feb	0.77	0.41	10.01	0.23	0.65	20.16
18-Feb	0.03	0.88	18.39	17.06	0.01	3.68
25-Feb	0.02	0.88	15.15	0.09	0.77	15.02
4-Mar	0.04	0.85	16.22	0.03	0.88	18.50
11-Mar	0.54	0.49	4.57	0.02	0.89	15.26

TABLE D13. Pixel count data (measured in kilopixels) taken each week during the experiment for Pahokee and Torrey muck soils, with the Hydra-Hume only treatment removed.

Soil	**Treatment**	**Feb. 4**	**Feb. 11**	**Feb. 18**	**Feb. 25**	**Mar. 4**	**Mar.11**
Torrey	Fertilizer	14.9 b	103 a	302 a	351 a	1005 a	1685 a
	HH & Fertilizer	16.8 a	96 a	271 b	340 a	1026 a	1659 a
Pahokee	Fertilizer	14.2 a	90 a	363 a	420 a	1396 a	2059 a
	HH & Fertilizer	14.9 a	95 a	356 a	413 a	1364 a	2108 a

* Treatments with different letters are significantly different at the p = 0.05 level.

Analysis of harvest data for individual soils

There were significant differences in fresh mass, dry mass and root vigor for both soils (Tables D14 and D15). Again all of the difference was a result of the Hydra-Hume without fertilizer treatment being much smaller than the other treatments (Table D14), except for root vigor in the Torrey soil were the Hydra-Hume plus fertilizer treatment was larger than the Hydra-Hume only treatment.

TABLE D14. Harvest data including fresh and dry mass, and root growth. There was no significant difference in % dry mass.

Soil	Treatment	Fresh mass (g)	Dry mass(g)	% Dry mass	Root vigor
Torrey	Fertilizer	12.4 a	0.82 a	6.63	1.63 ab
	Hydra-Hume	4.0 b	0.27 b	6.72	1.25 b
	HH & Fertilizer	12.2 a	0.82 a	6.83	2.38 a
Pahokee	Fertilizer	20.6 a	1.37 a	6.58	3.50 a
	Hydra-Hume	2.8 b	0.19 b	6.88	1.25 b
	HH & Fertilizer	22.3 a	1.56 a	7.01	3.38 a

* Treatments with different letters are significantly different at the p = 0.05 level.

TABLE D15. Statistical analysis of harvest data in Pahokee and Torrey muck soils, all treatments are included.

	--------------Pahokee----------------			----------------Torrey---------------		
	F Value	**Pr > F**	**Coeff Var**	**F Value**	**Pr > F**	**Coeff Var**
Fresh (g)	245.0	<.0001	9.1	15.5	0.0012	25.6
Dry (g)	71.1	<.0001	17.1	20.5	0.0004	22.2
%Dry	0.9	0.4400	10.2	0.5	0.6250	4.3
Roots	13.0	0.0020	25.9	3.26	0.0860	36.3

Analysis of harvest data for the fertilizer only and HH plus fertilizer treatments in the individual soils

When the unfertilized Hydra-Hume treatment was removed and data reanalyzed, all significant differences were removed (Table D16 & D17). However, the fresh mass in the Pahokee soil and the roots in the Torrey soil were approaching significance (shown in bold). The Hydra-Hume with fertilizer treatment was larger than the fertilizer only treatment in both cases.

TABLE D16. Harvest data including fresh and dry mass, and root growth, with the Hydra-Hume only treatment removed. There was no significant difference in % dry mass.

Soil	Treatment	Fresh mass (g)	Dry mass(g)	% Dry mass	Root vigor
Torrey	Fertilizer	12.4 a	0.82 a	6.63	1.63 a
	HH & Fertilizer	12.2 a	0.82 a	6.83	2.38 a
Pahokee	Fertilizer	20.6 a	1.37 a	6.58	3.50 a
	HH & Fertilizer	22.3 a	1.56 a	7.01	3.38 a

* Treatments with different letters are significantly different at the $p = 0.05$ level.

TABLE D17. Statistical analysis of harvest data in Pahokee and Torrey muck soils, excluding the Hydra-Hume treatment.

	------------Pahokee-------------			----------------Torrey---------------		
	F Value	**Pr > F**	**Coeff Var**	**F Value**	**Pr > F**	**Coeff Var**
Fresh (g)	**2.07**	**0.20**	**7.57**	0.02	0.90	22.35
Dry (g)	0.00	0.98	14.65	0.00	0.98	18.95
%Dry	0.96	0.37	10.37	0.77	0.41	4.87
Roots	0.06	0.82	21.20	**2.84**	**0.14**	**31.46**

Throughout the experiment it appeared that the plants were possibly nutrient limited so on the 40th day two of the four replicate plants in the fertilized treatments received an additional dose of fertilizer at 61 lbs/acre to see if the plants were under fertilized. This additional fertilizer slightly increased fresh mass of the fertilizer only treatment in the Torrey soil, but decreased fresh mass under all other conditions (Table D18). There were no statistical differences with additional fertilizer (data not shown).

TABLE D18. Fresh mass for additional fertilizer in fertilized treatments for both soils.

	Fertilizer	Fertilizer + Fertilizer	HH & Fertilizer	HH & Fertilizer + Fertilizer
Torrey	11.2	13.6	12.7	11.6
Pahokee	21.0	20.2	22.3	22.2

DISCUSSION

Lettuce did grow better in the Pahokee soil than in the Torrey soil throughout the study. More latent nutrients available in the Pahokee soil may have partially caused this (see soil analysis pg. 132). Presumably the preplanting fertilizer applied in this study would minimize any such effect. In addition the extra fertilizer added at the end of the experiment did not improve growth in either of the soils. If the smaller plant growth in the Torrey soil was caused by nutrient deficiency the plants should have responded to these fertilizer applications. Suggesting there are additional reasons for the improved plant growth in the Pahokee soil. In future work a higher initial fertilizer rate could help bring out any treatment effects.

There were no statistically significant effects of Hydra-Hume in this study. However, the addition of Hydra-Hume resulted in an 8.3% increase in lettuce fresh mass on the Pahokee soil ($p= 0.20$), and a 46% increase in root vigor on the Torrey soil ($p=0.14$). This implies that it might help lettuce growth in these muck soil. In order to see any improved plant growth the amount of Hydra-Hume should be increased in future research, especially considering the high levels of latent organic matter in these soils (not measured for this study but both soils were black like peat).